NDA 20-632

Knoll Pharmaceutical Company
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3 October 1996

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REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Summary

MERIDIA (sibutramine hydrochloride monohydrate) Capsules

Antiobesity agent - serotonin (5-HT) and norepinephrine (NE) reuptake inhibitors

Indications and Usage:
MERIDIA is indicated for the long-term treatment of obesity and should be used in conjunction with diet and exercise as part of a weight management program. MERIDIA should be used in patients with a BMI of ≥ 27 kg/m^2.

Regulatory Action: Approvable with Labeling changes (see p. 67).

Table of Contents

cc: Original NDA 20-632; HFD-24 JDeGeorge; HFD-400 JContrera; HFD-345; HFD-510 NDA 20-632; HFD-510 RSteigerwalt; DHertig; MHess

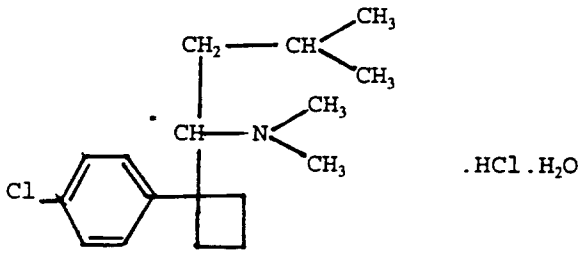
David H. Hertig
Pharmacologist

10/9/96

Related:

Supplier:
Drug Product - Knoll Pharmaceutical Company, Shreveport, LA

Formula: BTS 54 524



• Chiral Center

cyclobutanemethanamine, 1-(4-chlorophenyl)-N,N-dimethyl-α-(2-methylpropyl)-, hydrochloride, monohydrate, (±) $C_{17}H_{29}C_{12}.NO$ MW = 334.33

Dosage Form: Each MERIDIA capsule for oral administration contains 5 mg, 10 mg or 15 mg of sibutramine hydrochloride monohydrate (a white to cream crystalline powder).

It also contains as inactive ingredients, lactose monohydrate, NF; microcrystalline cellulose, NF; colloidal silicon dioxide, NF; and magnesium stearate, NF in a hard gelatin capsule [which contains titanium dioxide, gelatin, (5 and 10 mg capsules only), (5 and 15 mg capsules only) and other inactive ingredients].

Dosage: The recommended starting dose of MERIDIA is 5 mg administered once daily with or without food. If there is inadequate weight loss, the dose may be titrated up every two weeks in increments of 5 mg to a total of 20 mg. If there are no clinically significant changes in heart rate and/or blood pressure (see below) MERIDIA may be given in doses not to exceed 30 mg daily.

If patients develop diastolic blood pressures of ≥95 mmHg and/or heart rates of ≥105 bpm, the dose should be reduced.

Foreign Data: Yes - See individual studies.

Preclinical Studies:
, and Neurotoxic Assessment (13 Jun 96) review by Joseph F. Contrera, Ph.D. (HFD-900) - attached.
[BTS 62 930 is the + enantiomer of sibutramine BTS 54 524 - See also p. 45]

Pharmacology: [Additional Studies]
Cardiovascular effects -

BTS 54 524 at 1 and 3 mg/kg caused small but significant reductions in BP and HR at 1.5 and 5 hours after dosing to spontaneously hypertensive (SH) rats. BP and HR of normotensive rats were unaffected by 1 mg/kg; 3 mg/kg had no effect on BP but caused a significant bradycardia 1.5 hr. after dosing. 2/8 rats died after 3 mg/kg.

BTS 54 524 and two of its metabolites BTS 54 354 and BTS 54 505 at 3 mg/kg p.o. had no effect on BP and HR of (SH) rats but 4/8 rats died after each compound. The same dose caused no effect on BP in normotensive rats but caused a significant bradycardia at 1.5 hr. [It is reported that HR of rats under

conditions of test are normally 30% lower than unrestrained rats at room temp. - The phenomenon is described as thermal bradycardia appearing to result from restraint stress and a raised ambient temperature. - the finding here may not be a direct effect since the drug caused tachycardia when infused i.v. in pithed rats.] Activity of BTS 54 524 and these metabolites appear quantitatively and qualitatively similar.

Diuretic activity - Doses of 1-10 mg/kg BTS 54 524 orally were given to mice and rats in a water load of 30 ml/kg. In mice 3 and 10 mg/kg increased excretion of sodium and chloride (sig.). In rats these doses significantly increased urine vol., sodium and chloride excretion. Potassium excretion was not affected in either species.

3 mg/kg p.o. had little effect on urine volume in female water-loaded dogs. Although mean sodium and chloride excretions were greater in treated than vehicle treated dogs, increases were variable and not statistically significant. Potassium excretion was not affected.

Plasma Glucose - Hypoglycemic activity was not seen when 1, 2.5 and 10 mg/kg BTS 54 524 was given orally to glucose primed male Sprague-Dawley rats and blood glucose levels determined at 2 and 4 hours.

Immunology Screening - BTS 54 524 was inactive in immunology screening in the murine allogenic mixed lymphocyte reaction (MLR).

Pharmacological activity - The in vivo pharmacological activity of BTS 64 472 and BTS 64 473, respective fumarate salts of the aglycones of BTS 54 524 metabolites 5 and 4 were investigated. In man, BTS 64 472 (c-3-(1-amino-3-methylbutyl)-3-(4-chlorophenyl)-r-1-cyclobutanol fumarate) and BTS 64 473 (t-3-(1-amino-3-methyl-butyl)-3-(4-chlorophenyl)-r-1-cyclobutanol fumarate) have only been detected as conjugates and not as free species. Metabolite 5 was present in higher concentrations than metabolite 4. Both were inactive in the primary screen for antidepressants, the mouse reserpine reversal test. In additional tests for possible antidepressant activity (tetrabenazine-prevention and Porsolt test in mice and reserpine prevention in rats) BTS 64 472 showed considerably more activity than BTS 64 473. BTS 64 472 (but not BTS 64 473) induced ipsilateral circling in the unilateral nigrostriatal lesioned rat. Neither compound showed anticholinergic activity. The pharmacological activity of BTS 64 472 (greater than BTS 64 473) in vivo was considerably less than that of BTS 54 524 and the HCl salts of the secondary (Metabolite 1) and primary (Metabolite 2) amine metabolites of BTS 54 524. It is speculated that even if metabolites 4 and 5 were present in humans as free rather than conjugated entities, they would probably not make a large contribution to the pharmacological activity of BTS 54 524.

ADME and Pharmacokinetics:

Pharmacokinetic Summary: Studies have been carried out in mouse, rat, rabbit, dog, and monkey using the same strains and at similar dose levels to those used in the toxicity studies

[^{14}C]sibutramine hydrochloride was used as the radiotracer, supplemented on occasions with assays for specific metabolites. Rapid and extensive metabolism occurs precluding routine measurement of sibutramine, the parent compound.

Mice: male

Urinary excretion following a single 3.2 mg/kg dose of [^{14}C]sibutramine accounted for 49% of the labeled dose with 37% excreted in feces. Thus half of the dose was absorbed from the GI tract. 78% was excreted within 24 hours. Absorption was rapid with a Cmax of 720 ng equiv/ml at 0.5 hrs. A secondary

peak, attributed to enterohepatic recycling of biliary excreted radioactive material was ca. 280 ng equiv/ml at 4-8 hrs. Plasma radiolabeled material decreased to 6% of Cmax by 24 hrs.

Rabbits: female

Following a 3 mg/kg dose to female rabbits, urine was the major route of excretion (81%). 10% was recovered from the feces. 81% was recovered in 24 hrs. Absorption was rapid with peak plasma concentrations of 2110 ng equiv/g at 1.5 hrs. At 24 hrs. plasma concentration was ca.3% of Cmax.

Monkeys: male

Male cynomolgus monkeys given a 10 mg/kg dose showed the urinary label to account for 52% of the dose. 10% was fecal (0-144 hrs). 44% of the label was recovered in 24 hrs. Sacrifice at 168 hrs. showed low tissue concentrations. Cmax (4650 ng equiv/ml) occurred 2 hrs after dosing. Absorption was rapid with plasma concentrations of dose-related material being 72% of Cmax at 1 hr. Concentrations were 5% of Cmax at 24 hrs.

Another study with different males produced a Cmax of 5140 ng equiv/ml at 2 hrs with 1 hr. values of 79% of Cmax. Elimination from plasma to 3% of Cmax was seen by 24 hours.

Repeat Dose:

Rat:

3 mg/kg/day x 14.

Male - Daily excretion was relatively constant with cumulative values of 20.8% urinary and 65.0% fecal at the end of study. There was minimal plasma accumulation. Peak concentrations of radioactive material days 1, 7 and 14 were 690, 620 and 650 ng equiv/ml, respectively.

Female - Cumulative urinary excretion was 26.4% and fecal 56.6%. Peak plasma concentrations days 1, 7 and 14 were 600, 700 and 890 ng equiv/ml.

Pregnant Females - Beginning day 6 of gestation daily urinary excretion was 23.1% and fecal 65.7%. Peak plasma concentrations on days 12 and 19 were 880 and 970 ng equiv/ml.

Rabbits:

Pregnant - 3 mg/kg days 7-19 of gestation. Daily excretion was relatively constant. Day 19 urinary excretion was 68.9% and fecal 12.3%. Radioactive plasma profiles on day 19 gave a mean Cmax of 2610 ng equiv/ml at 1 hr. decreasing to about 3% of this value at 24 hours.

An additional group received another dose on Day 20: The mean plasma concentration of radioactive mater was 2650 ng equiv/g at 1.5 hrs and 160 ng equiv/g.

Dog:

1 mg/kg daily for 13 days. Daily excretion was relatively constant. At the end of study cumulative urinary values were 43.2% and fecal 46.7%. Plasma profiles were similar days 1, 7 and 13 with peak radioactive concentrations of 370, 510 and 550 ng equiv/g indicating a minor extent of accumulation.

Toxicokinetics: [See also Rosloff review p. 8]

Due to the rapid metabolism of sibutramine in animals and man, focus was on the primary pharmacologically active metabolites 1 and 2 and where practicable, on the inactive conjugated hydroxy-metabolites 5 and 6 which are found in humans.

Mouse: 13 week subacute study 3.2 and 50 mg/kg day.

Assay for metabolite 3, a substantial metabolite identified in mouse plasma. At the higher level, plasma concentrations of Cmax of 227 and 269 were similar for both single and repeated dosing. There was minimal accumulation. Concentrations were below assay limit at the lower dose.

Simulation of the CA study doses of 1.25, 5 and 20 mg/kg in the diet - plasma samples obtained at 6-hourly intervals during one day in week 4 and week 13 were assayed for metabolites 1 and 2. At all dose levels metabolite 1 concentrations were less than 0.5 ng/ml. Metabolite 2 concentrations increased with dose (not as proportionate) and were similar weeks 4 and 13 indicating maintenance of steady state. In males concentrations tended to be higher than in females. Cmax (ng/ml) and AUC (0-24h ng/h/ml) for the high dose at week 4 were males 9.5 and 175.8 vs females 5.3 and 89.4 and for 13 weeks males 10.9 and 159.0 vs females 5.3 and 75.0.

Rat: 6-month toxicity satellite groups:

Metabolite 2 was assayed from samples obtained on days 0, 29, 92 and 183. Doses were 3.2 and 20 mg/kg. Absorption was good. There was evidence of some accumulation at the lower dose level, but minimal accumulation at the higher dose level. Average Cmax (ng/ml) and AUC (0-24 hr ng·h/ml) for the 1, 3 and 6-month time points at 3.2 mg/kg (265, 298, 502 and 2490, 3290, 5560) were ca 4 times higher at 20 mg/kg (1229, 1736, 1792 and 8750, 15160, 13810).

Simulation of the CA study doses of 1, 3, 9 mg/kg in the diet were assayed at weeks 5 and 14 for metabolites 1 and 2. Although less than proportional, metabolite concentrations increased with dose level. Data at weeks 5 and 14 were somewhat similar. Concentrations in females tended to be higher than those in males. Cmax (ng/ml) and AUC (0-24h ng/h/ml) for the high dose at week 5 were males 2.3 and 30.6 vs females 6.9 and 94.2 and for 14 weeks males 1.4 and 23.4 vs females 7.2 and 122.4 for Metabolite 1. For Metabolite 2, Cmax (ng/ml) and AUC (0-24h ng/h/ml) for the high dose at week 5 were males 217.6 and 3780.0 vs females 292.3 and 4737.0 and for 14 weeks males 229.5 and 4183.8 vs females 430.3 and 7931.4.

Metabolites 5 and 6 aglycones - 14 daily oral doses of a 1:8 mixture at levels of 1, 3, 10, 20 mg/kg daily. One hour after the final 20 mg/kg dose the mean concentration of metabolite 5 was 1110 ng/ml and total metabolite 6 was 660 ng/ml giving a 1.7:1 ratio. A second study gave 1370 and 1300 ng/ml for a ratio of 1.1:1.

Rabbit:

Teratogenicity study satellite groups received 13 doses of sibutramine HCl at 12 or 24 mg/kg/day. Plasma samples at various times on days 6 and 18 were assayed for metabolites 1, 2, 5 and 6. The dose was taken up rapidly after the first or last dose. Reported that there were considerable inter-animal variations, but no apparent consistent change in metabolite AUCs showing minimal accumulation on repeated dosing.

Monkey:

Single oral dose 1, 3, 10, 20, 50, 100 mg/kg At 1.5 hrs. plasma assayed for metabolites 1, 2, 5 and 6. There was large interanimal variation but concentration generally increased with dose.

7-Days repeated dosing at 10 to 30 mg/kg with plasma taken at 1.5 or 24 hrs indicated minimal accumulation on repeated dosing.

Blood samples (up to 27 weeks) from 1-year toxicity study - see under 1 yr. study.

Distribution:

Distribution and persistence of radiolabeled material in tissues have been investigated following oral [^{14}C]sibutramine hydrochloride to mouse, rat (albino and pigmented), rabbit, dog and cynomolgus monkey.

Protein binding:

Equilibrium dialysis was used to investigate the extent of binding of metabolites 1 and 2 to plasma of mouse, rat, rabbit, dog, cynomolgus monkey and man. Studies were carried out at concentration ranges of these metabolites

[text continued on p. 8]

Sibutramine was rapidly metabolized in animals and man, and was rarely detectable in plasma as a result of the large first-pass effect. Drug exposure was, therefore, examined during toxicity studies by monitoring plasma concentrations of the pharmacologically-active metabolites, 1 and 2.
[Single administration plasma concentrations see p. 10]

The following tables were adapted from those of the sponsor:
Steady-state plasma data for pharmacologically-active sibutramine metabolites 1 and 2 in animals during repeated-dose toxicity studies and in obese human subjects

Species	Dose	Metabolite 1		Metabolite 2	
	mg/kg po	Cmax	AUC	Cmax	AUC
Mouse	20[a]	<0.5	-	7.8	125
Rat	9[a]	4.5	67.7	293	5159
Rat	20	[211]	[766]	1586	12570
Rabbit	24	6.1	-	148	592
Monkey	10	5.9	-	25.8	128
Man	0.15[b]	4.9	48.1	12.0	142

Units: Cmax, ng/ml; AUC, ng.h/ml
[a] administered in the diet
[b] based on a 15-mg dose to obese patients
- not calculable
[] single dose

Additional information was obtained by monitoring plasma concentrations of the conjugated hydroxy-metabolites, 5 and 6, which were present in substantial amounts in human plasma.
Steady-state plasma data for sibutramine conjugated hydroxy-metabolites 5 and 6 in animals during repeated-dose toxicity studies and in obese human subjects.

Species	Dose	Metabolite 5		Metabolite 6	
	mg/kg po	Cmax	AUC	Cmax	AUC
Rabbit	24	517	1660	1730	6930
Monkey	10	227	1830	888	4140
Man[a]	0.15	35.2	316	35.6	432

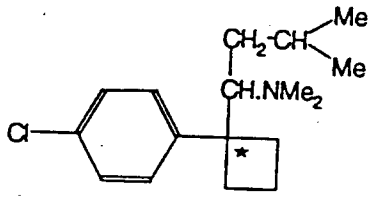
Units: Cmax, ng/ml; AUC, ng.h/ml
[a] based on a 14-mg dose to obese patients

Exposure to sibutramine and its pharmacologically-active metabolites 1 and 2 in animals during toxicity studies relative to exposure in patients

Species	Dose	Relative exposure			
		Dose	Metabolite 1		Metabolite 2
	mg/kg po		Cmax	AUC	Cmax	AUC
Man	0.15[a]	1	1	1	1	1
Mouse	20[b]	133	-	-	0.7	0.9
Rat	9[b]	60	0.9	1.4	24	36
Rat	20	133	43	16	132	89
Rabbit	24	160	1.2	-	12	4
Monkey	10	67	1.2	-	2	0.9

[a] based on a 15-mg dose to obese patients (once daily for 15 days)
[b] administered in diet
- not calculable
[Calculations based on total concentrations in plasma. Clearance appeared to be faster in males than in females. Binding to plasma proteins was reported to be sufficiently similar in animals and man.]

Structures of the major metabolites of sibutramine



Sibutramine

found in toxicological and clinical studies. The mean percentage bound for metabolite 1 ranged from 60% in rabbit to 94% in dog and 94% in human plasma; values for the other species were rat (78%), mouse (91%) and cynomolgus monkey (92%). Binding for metabolite 2 was 90% or more in all species studied i.e. cynomolgus monkey 90%, rabbit 91%, rat 93%, mouse 95%, and dog 96%. Metabolite 2 was 94% bound to proteins in human plasma. In general binding was independent of metabolite concentration.

Mouse:

Following a single oral dose of 3.5 mg/kg [^{14}C]sibutramine hydrochloride, whole-body autoradiography of male mice at 0.5, 6 and 24 hr. showed rapid uptake and distribution into the tissues. One-half hour after dosing, high levels of radioactive material were detected in the intestinal contents, stomach contents, kidney medulla, liver, brown fat, submaxillary gland, lung, parotid gland and Harderian gland. Clearance of drug-related material was seen in all tissues by six hours. At 24 hours radiolabeled material was still detectable - mainly in the intestines, and to a lesser extent, in the liver, lung and Harderian gland.

Rat:

Male and female albino and pigmented rats subjected to whole-body autoradiography at 0.75, 6 and 24 hours after administration of a single oral dose of 3 mg/kg [^{14}C]sibutramine hydrochloride showed a rapid and general distribution throughout body tissues. High levels of localized radiolabeled material were seen in the stomach and small intestinal contents, lung, kidney and liver, and in the uveal tract of the pigmented eye indicating melanin binding. By 24 hours the majority of material was cleared with the highest concentrations remaining in the pigmented eye and Harderian gland. Except for the pigmented eye, no sex or strain differences were detected.

A radioassay study was conducted with albino and pigmented rats with tissue measurements up to 336 hours following a single oral dose of 3 mg/kg in male rats. With the exception of pigmented tissues both strains showed similar concentrations. The highest were observed after one hour in the lung (11 μg equiv/g, albino; 9 μg equiv/g, pigmented), liver (6 μg equiv/g, albino; 5 μg equiv/g, pigmented) and kidney (3 μg equiv/g, albino and pigmented) relative to levels in plasma of 0.9 and 0.7 μg equiv/ml, respectively and in the eye of 0.2 and 4 μg equiv/g, respectively at 6 hours. By 168 hours tissues were cleared except for liver (<0.5 μg equiv/g were detected) and the pigmented eye. Binding in the pigmented eye was reversible as indicated by 97% of the peak concentration of radioactive material being cleared at 336 hours after dosing.

Pregnant albino rats were examined 0.75 to 24 hours after a single oral dose given on day 18 of gestation. The majority of maternal tissues showed a rapid uptake with localized areas of high radioactivity in the liver, lung, spleen, pancreas, stomach and small intestinal contents, salivary glands, Harderian gland, preputial gland and brown adipose tissue. Lower levels were observed in fetal tissues with no particular organ concentration. With the exception of maternal Harderian gland, neither maternal nor fetal tissues showed any persistence of radiolabeled material 24 hours after dosing. Pregnant and non-pregnant rats showed no apparent differences in tissue distribution of radioactive material.

Rabbits:

Female rabbits received a single oral dose of 3 mg/kg [^{14}C]sibutramine HCl. The Cmax was 2.1 μg equiv/g at 1.5 hours. The highest tissue radioactive levels at 24 hrs. were in bile (25.6 μg equiv/g); liver (1.1 μg equiv/g), lung (0.5 μg equiv/g), kidney (0.5 μg equiv/g) and intestines (0.2-0.4 μg equiv/g); the plasma concentration had decreased to 0.1 μg equiv/g. Remaining tissues contained <0.1 μg equiv/g. At 240 hours no detectable tissue concentrations remained except for liver (0.2 μg equiv/g), lung (0.04 μg equiv/g), kidney (0.03 μg equiv/g) and plasma (0.01 μg equiv/g).

Dog:

3 mg/kg [^{14}C]sibutramine HCl produced a peak plasma concentration of 1 μg equiv/ml at 1.5 hrs. in male dogs. Male dogs showed the highest radiolabel concentrations in bile (551.4 μg equiv/ml, CSF (28.8 μg equiv/ml), liver (21.8 μg equiv/g), lung (4.1 μg equiv/g), kidney (3.8 μg equiv/g), adrenal (2.3 μg equiv/g) with a plasma 1.2 μg equiv/ml - indicating rapid tissue distribution. Radiolabeled material was cleared from tissues by 216 hours with the exception of low levels in liver (0.2 μg equiv/g) and eye (0.1 μg equiv/g - associated with uveal tract, indicative of melanin binding.

Similar results were obtained with female dogs.

Cynomolgus monkeys:

Dose - single 10 mg/kg oral [^{14}C]sibutramine HCl. Measurement at 168 hours showed radioactive material in low concentrations i.e. less than 0.1 μg equiv/g in the majority of tissues, with higher levels in gallbladder/bile (2.15 μg equiv/ml), pigmented part of eye (0.88 μg equiv/g) and liver (0.23 μg equiv/g).

Repeated dose Studies:

Rat:

Male and female albino and pigmented rats - 2 mg/kg orally of [^{14}C]sibutramine HCl for 28 days samples at 0.75 , 6 and 24 hrs. after the final daily dose. At 45 min and 6 hrs radioactivity was localized in lung, liver, kidney, spleen, adrenal, Harderian gland, lymph nodes, preputial gland and salivary glands, with highest levels in stomach and intestinal contents and pigmented eye. Except for the pigmented eye by 24 hrs. radiolabel had declined markedly and there were no major sex or strain differences.

Measurements were made for up to 24 hrs. after 1 or 14 daily oral doses of 3 mg/kg labeled drug and 168 hrs after the last dose. The label was taken up rapidly and distributed into the tissues. For most tissues plasma ratios were >1. Plasma and many tissues showed a secondary peak from 2-12 hrs after dosing due to enterohepatic recycling. Steady-state was reached by the last dose and tissue concentrations increased by up to ca 2 times. The distribution pattern at steady state was similar to that after the first dose with that in females being greater than in males. At 0.5 hours after the last dose plasma concentrations were 0.4 μg equiv/ml for males and 0.9 μg equiv/ml for females. The majority of tissues in male rats had peak concentrations at 8 hrs. post dose, whereas almost all tissues from females peaked at 0.5 hours. In general tissue concentrations in female rats were higher by up to a factor of three. Radioactivity was promptly cleared from the tissues with concentrations generally less than 0.4 μg equiv/g at 168 hours after the end of dosing.

Pigmented male rats received 2 mg/kg orally for 1, 7, 14, 28 days. Radioactivity was measured in selected tissues 24 hours after the last dose. After the first and last doses, concentrations were measured for up to 12 weeks after dosing. Steady state was reached in 7 days except for the eye which took 14 days. Repeat dosing produced ca a 2-fold accumulation in tissues. The radio label cleared from tissues by 7 days following a single dose except for the eye which took 14 days. After 28 doses only the eye had measurable radioactivity (0.5 μg equiv/g) one week after dosing; these were not measurable by week 7.

3 mg/kg oral doses were given days 6 to 12 (7 doses) and 6 to 19 (14 doses) of gestation. Tissue levels were measured for up to 24 hours. Concentrations were reported to be similar after 7 and 14 days. A secondary peak was observed in plasma and some tissues 4 to 12 hours after dosing. The peak plasma concentration of radioactive material was 1 μg equiv/ml at 6 hrs. Most tissues had a tissue:plasma ratio >1. Fetal tissue levels (1.2 μg equiv/g) were similar to maternal plasma levels, but were low after 14 doses compared to maternal lung/liver and placenta. Pregnant and non-pregnant female rats showed no major differences.

Dog:

Tissue levels of radioactivity were measured in male and female dogs at the time of peak plasma concentration (1.5 hours) at the end of 14 daily 1 mg/kg oral doses of [^{14}C]sibutramine HCl. All tissues examined showed radioactive material with the highest levels found in male and female bile, liver, small intestine, lung, eye and kidney. Plasma concentration was 0.5 μg equiv/ml for both sexes with the pattern of distribution of drug related material similar in males and females. Radio labeled material in the eye was associated with the uveal tract.

Biotransformation:

Sibutramine HCL metabolism has been investigated after oral doses to mouse, rat, guinea-pig, rabbit, dog and monkey. The later studies focused on the major metabolites found in man namely metabolites 1, 2, 5 and 6. The trans isomer of metabolite 5, which was found as a very minor metabolite in man, was designated metabolite 4.

Rearrangement of Sponsor's Table:

Relative proportions of sibutramine and its identified metabolites in plasma

% of total identified	Metabolite					
material in:	1	2	3	5	6	Sibutramine
Mouse						
Dog						
Guinea-pig						
Rabbit						
Rat						
Monkey						
Man						

[]: limit of measurement a: mainly as aglycone NM: not measured

From Sponsors table:

Summary of plasma concentrations of sibutramine and metabolites observed in various animal species after a single oral dose of [^{14}C]sibutramine hydrochloride

Species	Dose mg/kg	Concentration (ng/ml)[a]					
		Parent	Metabolites 1	2	3	5	6
Mouse	50						
Rat	20						
Guinea-pig	30						
Rabbit	75						
Dog	10						
Monkey	10						

NM: Not Measured
a: Samples obtained at 1 or 1.5 h, after dosing
b: Mainly as aglycone

Mouse:

Metabolism was extensive one hour after a single 50 mg/kg oral dose of [^{14}C] sibutramine hydrochloride. Up to 15 radiolabelled components detected accounted for 58% of the radioactive material in plasma. There was no obvious major metabolite and sibutramine was only a minor component at 3% of the total plasma radioactive material. Metabolites 1, 2 and 3 were present and demethylation and oxidation were the major metabolic pathways. In another experiment a further 20% of the radiolabelled material was extracted.

Rat:

Metabolite 1 was the primary metabolic product in an in vitro rat liver study. Metabolite 1 was detected in plasma along with a major metabolite 2 after 30 mg/kg. Metabolite 3 but not 1 and 2 was found in the urine. Demethylation was a major phase 1 route of metabolism with metabolite 2 accounting for ca 54% of total radioactivity in pooled plasma 1 hour after a single 30 mg/kg dose.

A single 20 mg/kg dose of [^{14}C]sibutramine was given to male rats metabolites were accounted for and in addition radiolabeled material present in pooled brain tissue was investigated. About 90% of the drug related material in rat brain was non-conjugated metabolites, mainly metabolite 2 and some of metabolites 1 and 3. No unchanged parent drug was observed.

Following a single 3 mg/kg dose biotransformation to $^{14}CO_2$ did not occur.

Guinea-pig: males

Metabolism was extensive after a single oral dose of 30 mg/kg with 46% occurring as 11 components with metabolites 1 and 2 as major components and sibutramine a minor component.

Rabbit: females

A large number of metabolites were detected following a single oral 75 mg/kg dose with sibutramine being only a minor component.

Dog:

Male dogs were given a single oral 10 mg/kg dose. Low levels of sibutramine and metabolite 1 were detected as well as low levels of the aglycones of metabolites 5 and 6.

Monkey:

A 10 mg/kg dose was given to male cynomolgus monkeys. They were redosed 72 hrs later when the concentration of radiolabel was less than 1% of peak and a 1.5 hr. sample taken. About 12 components were present including very low levels of metabolites 1 and 2 and sibutramine. Enzyme hydrolysis showed 12 aglycones including metabolite 5 and 6. Thus, sibutramine appeared to be rapidly and extensively metabolized in the monkey. Brain tissues were also taken 1.5 hours post dose. About 87% of total radioactive material was extracted prior to hydrolysis and comprised six components, including metabolites 2, 3 and the aglycone of metabolite 6; sibutramine was not detected.

The high degree of conjugation of plasma metabolites was confirmed in a similar study. 31% of urinary activity prior to hydrolysis gave up to 14 peaks including the aglycones of metabolite 5. Enzyme hydrolysis extracted a further 30% composed of up to 16 peaks including aglycones of metabolites 5 and 6. Acid hydrolysis gave a further 14%. None of the three urine fractions gave any major metabolites.

In monkeys there appeared to be minimal stereoselectivity in the primary metabolism although there was some stereoselective handling of secondary metabolite enantiomers by the cynomolgus monkey dosed orally.

Rat - Effect on Drug Metabolizing Enzymes:

20 mg/kg sibutramine was given to male rats for 7 days. Comparison with controls indicated that sibutramine appeared to have a minimal effect on total cytochrome P450's, especially CYP1A1 and CYP2B compared with the known inducing agents, phenobarbitone and ß-naphthoflavone.

Toxicity Studies:

Inhalation - Rat:

Five per sex Charles River CD rats were exposed to sibutramine concentrations of 0.017, 0.063 and 0.431 mg/l by nose only inhalation over a 4 hr. period followed by a 14 day observation period. Tail-tip injuries in the majority of the high dose group appeared to be self inflicted. One had to be killed due to self inflicted injuries. The two higher doses showed staining of the fur, piloerection and marked behavioral changes, such as exaggerated startle response, seeking behavior, and aggression. Signs of ejaculation were also seen. Some piloerection and ejaculation were seen in the low dose. The high dose animals lost body weight which remained less than that of controls until study termination. The mid-dose animals lost body weight transiently. Some high dose showed rhinitis and hepatocellular necrosis or vacuolation and in the one that died lung congestion and edema.

Monkeys - Sequential oral:

Sequential oral (gavage) doses between 1 and 100 mg/kg were administered to 6 pairs each of 1M;1F cynomolgus monkeys either as a single dose or as seven-day repeat doses (Report TX89010).

Single doses: 1, 3 and 10 mg/kg showed no overt signs. Pupils were dilated at 20 mg/kg. Excitability and pupillary dilation were noted at 30 mg/kg and additionally at 50 and 100 mg/kg there were signs of increased activity with signs of visual disturbance in one. Transient reductions in bodyweight and food consumption followed doses of 10 mg/kg and above. Glucose values were sometimes low and ALT, AST, bilirubin, triglyceride and urea transiently increased at 30 mg/kg and above (but inconsistent and not dose related). There was no effect on hematology or ECG. No definite histopathological abnormalities were evident however, changes of hepatocellular fat vacuolation and thyroid congestion, possibly stress/agonal related, were seen in the one male each that died at 50 and 100 mg/kg.

Seven-Day Repeat Dose: At 20 and 30 mg/kg there were signs of CNS stimulation -increased activity and excitability- and visual disturbance in the male after 30 mg/kg doses. All levels showed pupil dilation. The 30 mg/kg pair were killed due to severity of signs and humane reasons. After 4 days the 20 mg/kg pair were observed for recovery and dosed at a lower level (15 mg/kg). The 15 mg/kg pair showed pupil dilation (also 10 mg/kg pair) and increased activity. Some alterations but no consistent changes were seen in hematology and blood chemistry. ECG and bone marrow were not affected. Organ weights were normal and hepatocellular fat vacuolation was seen at 20 or 30 mg/kg.

Three-Week Oral Toxicity of BTS 54 524 in the Mouse:

Report TX87009 Mar 1987. Batch 1/R4 as P24/59. To establish dose levels for a 90-day study.
Q.A.: Signed QA Manager as in compliance with FDA GLPs (no inspection dates).

10/sex/group Charles River CD-1 mice were given BTS 54 524 orally by gavage at doses of 0, 6.25, 12.5 or 25.0 mg/kg. Histopathology was conducted on controls and high dose and livers of low dose.

Sibutramine produced (mainly at the two higher dosages) increased activity and increased food consumption, lower liver weights and liver glycogen disturbances (loss or uneven distribution) in the liver lobules probably consequential to CNS stimulant activity. Changes were dose related in degree. Mid- and high dose males lost weight or showed reduced weight gain after the first dose after which growth rate was comparable to controls. Females showed significantly lower mean group weights than controls day 3 and on various

occasions during the first two weeks at the higher dosages. At later times weights were comparable to controls.

It was decided that a wider range of dosages should be used for the 13-week study.

13-Week Oral Toxicity Study of BTS 54 524 in the Charles River CD-1 Mouse:
Report TX88022 Study Code MC 0029
Feb 1988. Batch: No. 2 as P21/37 Q.A.: Present

Dose: 0, 3.2, 8, 20, 50 mg/kg daily for 13 weeks
Vol. 0.1 ml/10 g bodyweight

No. Animals: 12M;12F per group

Results:

There were two deaths one male at 8 mg/kg (day 56), probably due to dosing trauma and one male at 50 mg/kg killed (Day 14) due to fighting injuries. Observations included behavioral changes characteristic of CNS stimulation (most pronounced during first 6 hrs. after dosing, but remaining up to 24 hrs. in a few) for both
sexes which included increased activity, vocalization, excitability and less commonly, tenseness on handling as well as increased aggression in males. Beginning at 8 mg/kg and lasting throughout the treatment period, these findings were dose-related in incidence and severity. At 50 mg/kg several mice had rales, slow or uneven respiration and sneezing (also one 20 mg/kg male). At this dose there was also a high incidence of injuries due to fighting in males - as a result one had to be killed. Isolated incidences of increased vocalization was evident for some controls and low dose.

20 and 50 mg/kg females showed a slight reduction in bodyweight gain. High dose males were similar to controls with slight gains over controls being seen in the other treated groups.

Food consumption was not affected by treatment.

The slightly lower group mean myeloid:erythroid ratios (control and high dose) for high dose males and females were within normal and not significantly different from controls.

Clinical chemistry was not done.

High dose males showed a slightly elevated but statistically significant ($p < 0.05$) spleen weight without microscopic change. One 8 mg/kg animal had an unusually large spleen associated with findings of mild hemopoiesis and poor white pulp development.

Histologically at 8 mg/kg and up livers showed periportal glycogen loss, with a few mice showing reduced glycogen throughout the liver or slight centrilobular hepatocyte enlargement. Compared to controls, salivary glands of treated females showed a higher incidence (but within range of that generally seen in control males) of cytoplasmic vacuolation in the acinar cells however, only a few mice at 3.2 mg/kg were affected. Electron microscopy of the acinar cells showed vacuoles in treated to be no different from those in controls. [It is reported that acinar cell vacuolation is common in male mice (but less so in females), and was present in all males examined, treated and control.

Based on behavioral changes the sponsor concluded that 20 mg/kg was the highest appropriate dosage to be used in the oncogenicity study.

6-Month Oral Toxicity Study of BTS 54 524 in Charles River CD Rats:
Report TX86091 Nov 1986.
Batch/Lot: No. 2, as P21/37 Q.A.: Signed QA Manager as incompliance with FDA GLPs (no inspection dates).

Dose: 0, 3.2, 8, 20 mg/kg/day orally by gavage for 6 months.
Vol. 0.5 ml/100 g bodyweight

No. Animals: 20M;20F per group.
Additional 16M;16F control and high dose for 1 mo recovery period.

Histology was conducted on all tissues of rats that died preterminally, control and high-dosage killed after 6 months, livers and lungs from lower dosage and off-dose (recovery) groups, and macroscopically abnormal tissues.

Results:

There were 6 deaths, three controls, 1 low dose and 2 high dose treated animals. Death in 1 low dose female day 26 appeared to be due to dosing trauma. One high dose male was killed on day 121 due to a deteriorated condition and one female was found dead on day 136 apparently due to faulty intubation.

Behavioral changes consistent with CNS stimulation included the following: vocalization, excitability, increased salivation, increased activity and tenseness (and occasionally aggression) on handling. Most males at the highest dosage were affected but rarely showed signs after the 5th week. Effects occurred at all dosages in females and were dose-related in incidence and severity and lasted throughout the treatment period for the high dose. Salivation was increased more frequently in both high dose males and females during handling. During the latter part of study 2M;1F high dose had swollen hindlimbs typical of arthritic changes sometimes seen in aging rats. During the recovery period, treated animals occasionally still showed vocalization and tenseness on handling.

Bodyweight gain of both sexes was reduced in a dose related manner for all dosages during the treatment period (most weekly group mean values were significantly lower than appropriate controls). By the end of the recovery period females were still slightly lower while males had regained their weight deficit.

Food consumption was reduced during the first week, after which males ate slightly less and females slightly more than controls. During the recovery period both males and females ate more than controls and increased weight. By the end of the recovery period females were still slightly lower but males had regained their weight deficit.

Ophthalmoscopy showed no treatment-related effects.

Hematology showed significant changes of slightly higher hemoglobin and PCV values and lower platelet counts with increased MCV and MCH values for high dose males in the early part of study. At the end of dosing, there were no clear cut differences from controls. At the end of the recovery period, treated male MCH and MCV values were significantly higher (but with a minimal difference) than controls. Blood samples from several high dose females and a few mid-dose females were either difficult to obtain from the tail vessels or clotted during collection. Various other findings, probably not treatment related, included higher neutrophil counts in high dose females at the end of treatment but not at this time period in the corresponding high dose group that went on to recovery. Lymphocyte counts, significantly reduced in treated females at the end of the recovery period, were within normal limits. Other findings of statistically significant differences from controls were generally within normal range.

Terminal bone marrow smears showed all treated groups to have slightly increased myeloid:erythroid ratios. At the end of dosing all male groups showed a statistically significant increase in M:E with a corresponding decrease in total erythroid count, increases in the total myeloid count and for the high dose group neutrophil count. The picture was similar for mid- and high dose females with statistically significant increased total myeloid and neutrophil counts, and decreased late normoblast count. Although the M:E ratio was slightly increased and the total erythroid count was slightly decreased there was no significant difference from controls. After the recovery period, statistical differences in male groups showed no particular trend. Treated female mean values were similar to that after treatment, but individual values were within normal limits.

Treated rats tended to have reduced levels of serum glucose, urea, potassium, creatinine, triglyceride and protein. High dose males had lower calcium after 12 weeks and at the end of the dosing period females had lower bilirubin levels. A few treated animals had lower AST, ALT or ALP activities. Slight reductions were seen in mid- and high dose males and all female groups in serum albumin and increases in γ-globulin. After recovery there were no meaningful differences between BTS 54 524 and controls. The statistically significant differences for ALP activity and ß-globulin in males were reported to be within normal limits.

Urine specific gravity was higher in BTS 54 524 groups and was associated with a slightly reduced flow rate in females after 5 weeks. Specific gravity and electrolyte excretion rates were significantly different on several occasions. Urine concentrating ability was reported as appearing to be unimpaired. Mid and high dosed females showed potassium excretion and urine flow to be slightly reduced after 5 weeks and sodium excretion to be slightly increased after 12 and 26 weeks. At the end of the recovery period females showed slightly increased urine flow and electrolyte excretion.

After 6 mos. treatment, all levels showed slight significantly elevated salivary gland weights for females. The two higher dose females also had slightly but significantly elevated kidney weights. Kidney and salivary gland weights were normal at the end of the recovery period. Significantly elevated uterus weights for the two higher dosage levels at the end of treatment were still slightly higher than controls after recovery. BTS 54 524 males showed lower absolute levels for several organs which were normal relative to body weight reflecting effects on growth rate. At the end of the recovery period, there were no apparent differences for males.

Macroscopic findings were not considered to be treatment related.

Histology after 6 mos. treatment showed a higher incidence of centrilobular and midzonal hepatocyte vacuolation for all dosages of males. After the recovery period the change was still apparent but to a slightly lesser extent. After 6 mos. treatment high dose females had a higher incidence of subpleural macrophage aggregates in the lungs - but not after the recovery period. These foci were also seen in controls, but not in the intermediate group. Other organs appeared to be free of pathological drug related changes. It is reported that BTS 54 524 did not cause histologically-detectable ototoxic (right cochlea) damage (10M;10F control and high dose examined).

6-Month Oral Toxicity Study of BTS 54 524 in the Beagle Dog:
Research Report TX86080 dtd Nov 1986.
Batch: No.2, as P21/37
Q.A.: Signed QA Manager as in compliance with FDA GLPs (no inspection dates).

Dose: 0, 1, 3, 10 mg/kg daily for 27 weeks. Vehicle - purified water BP.

No. Animals: 4M;4F per group. Additional 4M;4F control and high dose for 4 wks. recovery. ca 5-10 mos old.
Males ca 10-16 kg; Females ca 7-13 kg.

Concurrently with this study another dog study (TX86082 - see below) was conducted to investigate oculotoxic potential. Controls were common to both studies. BTS 54 524 dogs were given a maximum tolerated dose (MTD) titrated throughout a 24-week dosing period. The initial 10 mg/kg dose was increased to 12.5 to 15 mg/kg [HTD currently 20 mg (for a 70 kg human = .28 mg/kg)]. These dogs were also examined for a range of conventional toxicological parameters.

Results:

Stereotyped movements (licking, nodding, head arching, paddling, running in and out and side to side) were occasionally seen in a few animals at 1 mg/kg

up to week 11, throughout treatment for some at 3 mg/kg and in most 10 mg/kg dogs. Other signs seen in high dose dogs and occasionally at 3 mg/kg included increased activity, excessive panting, increased salivation, occasional vomiting, abnormal gait and posture, dilated pupils, reduced food consumption and a tendency to eat less readily than usual, also bodyweight loss. From week 6 on some high dose dogs appeared at times to be unresponsive to the presence of people. By the end of the day most overt signs had usually disappeared, although often pupils were dilated 24 hrs after dosing. Stimulatory effects became less pronounced as treatment progressed; behavior returned to normal from the day that treatment stopped. At weeks 6 or 13 high dose dogs had slow or incomplete pupillary light reflexes (also 2 low dose and one mid-dose dog) and rectal temperatures were slightly lower. Some pale gingivae were evident at week 13 and some were still evident at week 27. At the end of the recovery period 1 high dose female still had incomplete pupillary light reflexes and became excited upon handling. By week 27 these effects had mostly disappeared and they were not apparent after the recovery period.

One low dose F had cachexia, scapular sores and left eye corneal ulcer and a reddened conjunctiva at week 13. Its condition was markedly improved by week 27.

Several animals lost weight during the treatment period, especially 1F control, 1F low dose, 1F mid dose and 2M;3F high dose. Weight was lost rapidly in some and more gradually in others. Weight stabilized in those that lost weight and that did not have food increased. Dogs remained stable or gained weight during the recovery period (Control -.2 to +.3; high dose +.1 to +2.2kg).

Food ration was increased about half-way through treatment for some of the high dose animals that lost weight as well as 1 each control and low dose - marked bodyweight improvement followed. Several high dose and one mid-dose generally ate less during the first two weeks. There was no effect on food during the recovery period.

Treatment showed no consistent effects on electrocardiogram [1 low dose F and 1 high dose male had exaggerated sinus rhythm (and slower heart rates) week 13] or ophthalmoscopic examination or on hematological or myelogram parameters or on fecal occult blood.

Other inconsistent changes included 3 high dose dogs with slightly higher serum cholesterol levels at different occasions. Isolated increased ALP values over pre-dose were seen in some dogs (including 1 ea control and low dose). Thus, relationship to treatment is unclear. ALT was increased in 1 low dose female during weeks 6, 13, 27 and 1 high dose male during weeks 13, 27 and after recovery. One mid-dose male and one high dose female had higher serum ALT weeks 13 and 27.

Urine volume was reduced in some treated dogs during this period [mainly in high dose dogs that had eaten little or no food (and probably no water) the day prior to collection]. These reductions were associated with decreased excretion rates of sodium and/or potassium ions. At week 27, except for one high dose male, findings had returned to normal. Blood and hemoglobin in the urine was seen in a similar number of controls and treated.

Three high dose dogs showed enlarged livers after 27 weeks with the relative organ weight of two of them being slightly heavier (also one control female). After the recovery period, there was no apparent difference from controls. Various other differences showed no apparent treatment relationship.

Macroscopically four high dose livers appeared enlarged with an accentuated lobular pattern (also 1 high dose at recovery) in three.

Histopathology revealed no liver or any other tissue treatment-related pathology - thus recovery tissues were not examined. [It is stated that in the concurrent oculotoxicity study, a titrated maximum tolerated dosage (10 increasing to 15 mg/kg daily) for 24 weeks produced no treatment-related histopathological findings - see below.]

24-Week Study of BTS 54 524 - Oculotoxicity Study in the Beagle Dog:
Report TX 86082 Nov 86.

Batch: 2, as P21/37
Q.A.: Signed QA Manager as in compliance with FDA GLPs (no inspection dates).

Controls concurrent with above study (TX86080).

This study was conducted to investigate whether BTS 54 524 possesses oculotoxic potential in dogs, because it binds to melanin and accumulates in the uveal tract of the eye. Indirect ophthalmoscopy was conducted on all dogs pre-dosing, at four-weekly intervals during treatment and at the end of recovery. Fundic photographs were taken of all dogs pre-dosing, and at the end of treatment and recovery periods.

Dose: MTD: 10 increasing to 15 mg/kg daily orally by gavage for 24 Weeks. [10 mg/kg increased to 12.5 mg/kg from day 57 and to 15 mg/kg from day 71] Vol. 2 ml/kg solution in purified water B.P.

No. Animals: 8M;8F 4M;4F were retained for a 5 week recovery period. 8M;8F controls received vehicle and 4M;4F retained for the recovery period. ca 10 mos. old - Males ca 9.5-16 kg Females ca 7-12 kg

Results:

Prolonged pupil dilation and inhibition of the pupillary light reflex was seen, however, it is reported that there was no evidence of oculotoxicity. Other treatment related signs included increased activity, stereotyped movements, excessive panting, increased salivation, occasional vomiting, abnormal gait and posture, reduced food consumption and a tendency to eat less readily than normal, reduced urine volume, bodyweight loss, and slightly reduced rectal temperature. Increased activity was particularly noticeable up to week 6 (10 mg/kg) as the dosage was increased dogs became less active and became unresponsive to the presence of people. Although pupils were often still dilated the next morning, the majority of signs were subsiding by the end of the day. Behavior returned to normal from the day treatment was stopped.

Except for 2M;1F BTS 54 524 treatment caused weight loss. Most dogs lost weight at the beginning of the dosage period with some showing further losses when the dose was increased to 15 mg/kg. Females were more susceptible than males.

Several treated dogs ate less during the first two weeks after which food consumption returned to normal in most dogs. In general treated ate food less readily than controls. Because of weight loss, food ration was increased by 100 g for 1 control and 1 treated female from week 15 and for two treated females from week 18. During the recovery period those that had been treated gained weight with gains tending to be greater in those that had lost more.

Ophthalmoscopy and fundic photography showed no apparent treatment related effects. Minor, common variations in fundic appearance seen in treated and control were considered to be incidental. Recovery had no effect. Unilateral increased ocular pressure in one dog was of doubtful clinical significance.

Hematology and electrocardiograms showed no consistent treatment related effects either after treatment or recovery.

Three treated dogs had isolated increased serum ALP values - treatment relationship was unclear since these changes were inconsistent.

Urine volume was considerably reduced for 5M;2F week 14 but was low only in 3 of the males during week 24. These reduced rates were reported to be associated with decreases in the excretion rates of sodium and potassium and coincided with these dogs eating little or no food the preceding day. 1M;1F control also had low sodium excretion rates but normal urine volumes. After recovery urinary volume and sodium and potassium excretion values had returned to normal for the 2 males affected at week 24. Raised urinary AST activity of 1F week 24 and 2 recovery females were not consistently confirmed in samples taken later in the week. In general similar numbers of control and treated showed blood and/or hemoglobin in the urine.

At the end of the treatment period two BTS 54 524 dogs had slightly elevated liver weights - myelograms showed one of these and one other dog to have slightly higher myeloid:erythroid ratios. After recovery one BTS 54 524

dog had a slightly elevated liver weight - myelograms were normal. Other organ weights showed no obvious treatment relationships.

Macroscopic examination showed one male treated to have an accentuated lobular liver pattern at recovery.

No apparent treatment related histopathology was seen at the end of the treatment period, therefore the recovery group was not examined.

Pilot Study in Cynomolgus Monkeys:
Report 7080; No. 643548 (completed Nov 1989). [Boots Report TX90017 dtd Mar 1990] Batch: No. 4. Q.A.: Present.

Objective was to determine the maximum tolerated dose of BTS 54 524 during daily oral treatment for 7 days.

Dose: 10, 15, 12.5 (introduced after completion of Gps 1 & 2) mg/kg/day for 7 days for Groups 1 & 2 and 5 days for Gp 3. 4 $ml \cdot kg^{-1}$ Purified Water BP.

No. Animals: 2M (2.6-3.4 kg) and 2F (2.7-3.4 kg) per group

Results:

Clinical signs were mainly pupillary dilation at all dosages and hyperactivity, excitability and stereotyped behavior at 12.5 mg/kg and 15 mg/kg with frequency and severity being dose dependent. Also seen at the two higher doses were lip smacking and tongue protrusion.

Body weight at 10 mg/kg was unaffected while at 12.5 mg/kg both monkeys lost 0.3 kg over the 5 day treatment period. At 15 mg/kg the female lost 0.5 kg during the 7 day treatment period but the male was not affected.

Food consumption, slightly reduced at the low dose, showed a marked reduction at the two higher doses (early only for the 15 mg/kg male).

Monkeys were disposed of without pathological evaluation.

The maximum tolerated repeat dose in Cynomolgus monkeys was determined to be 10 mg/kg.

13 Week Oral (Gavage) Toxicity Study of BTS 54 524 in The Cynomolgus Monkey, With a 6-Week Treatment Free Period: . Report 6099-316/20; TX90022. Mar 1990. Batch: No. 4 Study Began Dec 88. Q.A.: Present.

Dose: 0, 1, 3, 10 mg/kg/day for 13 weeks orally by gastric intubation. Vehicle - purified water BP Vol. 4 ml/kg

No. Animals: 4M;4F per group with an extra 4M;4F per group control and high dose for recovery. Macaca fascicularis monkeys. males 2.00-3.10 kg; females 1.70 to 3.00 kg.

Results:

On Day 16 one high dose male collapsed and died before daily dosing. Shortly before death it had no muscle tone and appeared dehydrated. Adrenals were moderately dark, there was a slight thickening of the esophagus at the stomach junction and there was a gaseous distension of the jejunum, ileum, caecum and colon and ca 20 ml yellow fluid in the stomach. The animal appeared to have been stressed - histology showed an area of fibrosis in the lamina propria of the esophagus at the cardiac sphincter, hyperplasia of the adrenal cortex and pituitary chromophobes, and marked involution of the thymus.

During week 6 one low dose male had a wound on the right cheek, probably self inflicted. The animal was kept off dose for 19 days to facilitate healing. During week 12 it again had a wound in the right check which was forming a large

pouch fistula. Since there was only one week of study remaining, the animal was sacrificed following collection of blood and urine samples. No treatment-related findings were noted post-mortem.

During the study there was a slightly increased incidence of vomiting. Five high dose monkeys vomited immediately after dosing between 1 and 31 occasions during the study and once in two control females. Other signs were common in wild-caught monkeys.

During the first 2 weeks of treatment most of the high dose monkeys showed body weight losses, after which changes were similar to that of controls. During the recovery period weight changes for the high dose were comparable to control.

Food consumption showed no treatment related-changes.

Ophthalmoscopy and Electrocardiography did not show any treatment-related abnormalities.

No effects of treatment were noted in hematology, blood chemistry, or urinalysis (urinary concentration was satisfactory). However, urine volumes of both M and F were increased for the low dose at 13 weeks.

Spleen weights of high dose monkeys tended to be lower than those of controls at the end of treatment but not after recovery. Although not statistically significant, the following were noted at the terminal kill: male gonad weights and gonad body weight ratios of the mid-dose were less than that of controls while that of the low and high doses were greater (due to 1 ea. very large in ea. of these groups) than controls; uterine weights, absolute and relative, showed a dose related decrease (but within range of controls). For the recovery animals absolute and relative weights of male gonads of the high dose (1M very high wt.) were also greater than controls and uterine absolute and relative weights were less than controls - no statistical significance was reported.

Necropsy findings showed no treatment-related changes. Findings were of a minor nature usually seen in wild-caught monkeys and included pale areas in the liver, minor lung lesions, ovarian cysts and intestinal nematodes.

Histopathological findings were reported to show no treatment-related findings. The incidence of immature testes, control through high dose, were as follows: 4,1,4,1.

In general no treatment-related changes were seen in 1 or 3 mg/kg/day monkeys.

52-Week Repeated-Dose Oral Toxicity of BTS 54 524 in Cynomolgus Monkeys: Report 7539, Project 643464 dtd. Dec 1990 .td Jul 1991] Batch: No. 4. Q.A.: Present.

Dose: 0, 1, 3, 10 $mg \cdot kg^{-1}$ for 52 weeks. Vehicle - purified water BP.

No. Animals: 5M;5F per group Cynomolgus monkeys

Pretrial and during weeks 13, 25 and 52: electrocardiography, ocular, hematology, clinical chemistry and urinalysis investigations.

Day 1 and one day during weeks 2, 4, 8, 15 and 27 serial blood samples were collected for pharmacokinetic studies. (Histology by the sponsor.)

Results:

During the first two weeks of treatment one male and one female high dose animal showed signs of behavioral stimulation. The male showed signs of hyperactivity and excitability at various times which included lip-smacking on day 12. The female showed subdued behavior and reduced activity on day 13 and signs of agitation and continuous lip-smacking on day 14. Neither of these animals showed treatment-related overt signs from the third week on.

One of these, a female, showed a marked reduction in food consumption and weight loss during the first 4 weeks after which weight stabilized but the initial loss was not regained. One control also showed reduced food consumption in week 4. No other treatment-related weight and food consumption findings were reported.

Ophthalmoscopic examination showed no abnormalities.

Electrocardiographic measurements showed no changes associated with treatment. Heart rate, interval data and wave forms were within normal ranges.

Hematology showed slight reductions in mean hemoglobin and red cell count for high dose females from week 25, however individual values were reported within normal ranges. Occasional white cell counts and platelet values were outside reference ranges - it is reported that such findings are routine in these monkeys. There was a marginal, but statistically significant increase in eosinophil count for male treated groups in week 25. The significance is uncertain since controls were at the lower end of normal.

Bone marrow examination showed no evidence of treatment-related effects.

Clinical chemistry showed a few animals including controls with occasional elevations in aspartate aminotransferase and alanine aminotransferase such as are routinely seen in these primates. The statistically significant differences in mean alkaline phosphatase (also females pretrial), creatinine (males) and triglycerides at various time periods (mainly in females) were reported to be within normal ranges. The significance of the statistically significant increases in triglycerides, potassium and calcium in high dose females during week 52 is uncertain.

Urinalysis revealed no apparent drug-related effects.

Fecal occult blood was negative.

It is reported that there was no change in organ weights related to treatment. There were statistically significant differences in mean kidney weights (corrected for body weight) in all male (decreased) treated groups and the high dose female (increased) group; however, individual values were reported within the species normal range. Although not statistically significant mean absolute high dose testis, prostate and uterine weights were decreased (see below).

Necropsy findings reported no apparent drug-related findings. The incidence of lung nodules was increased in treated as follows (control through high dose): Males 0, 3, 0, 3; Females 1, 2, 4, 1.

Histopathology also reported no treatment-related findings. Although not significant the incidence of adrenal accessory cortical nodules was reported increased over control for female treated. In one high dose both testes were small with one retained in the abdomen. Three high dose and 1 ea. control, low and mid- dose had immature epididymis and immature testes. The prostate was immature in 2 high dose and 1 ea. in the control and other treated groups.

Pharmacokinetic portion of study:

Plasma samples were taken at 1.5 hrs after the 1 mg/kg sample and 24 hrs. after the 10 mg/kg/day sample on day 1 and during weeks 2, 4, 8, 15 and 27. A limited plasma profile was also taken on day 1, week 4 and week 15 at the higher dose level.

Pharmacokinetic studies showed no metabolites 1 and 2 detected in the majority of samples at 1 mg/kg; measurable concentrations of metabolites 5 and 6 confirmed dose absorption. Metabolites 1 and 2 were detected in only a few samples 24 hrs. after the first dose of 10 mg/kg, though higher levels of metabolites 5 and 6 were detected. Apparent larger increases in plasma concentrations of metabolites 5 and 6 observed after repeated dosing for the lower dose group but not for the higher dose group are unexplained. The comparative exposure of the high dose group was a factor of at least 7 for metabolite 2, when using 1.5 hour concentration values. The relative exposure values for the aglycones of metabolites 5 and 6 were 5-10, and 8-40, respectively. Although inter-animal variation was apparent there were no consistent sex differences in metabolite plasma concentrations. 24 hours after

dosing week 27 plasma concentrations of metabolites 5 and 6 were not measurable. [Reported that plasma concentrations of total metabolites 2, 5 and 6 taken 1.5 hrs. after the 10 mg/kg dose on day 1 were similar to those found in a previous single dose radiolabelled study, although plasma concentrations of metabolite 1 were lower.] Rapid clearance of all metabolites from plasma did not permit calculation of any reliable pharmacokinetic parameters.

Reproduction Studies:

Fertility Study in Charles River COBS CD Rats:

Report TX87045. 510-010. dtd. April 1987. Batch: 2 as p21/37 O.C. No. 00669 B.
Q.A.: Present.

Dose: 0, 1, 3, 10 mg/kg/day orally by gavage Vol. 10 ml/kg
Males - 60 days prior to mating until sacrifice
Females - 14 days prior to mating until sacrifice
Controls deionized water only.

No. Animals: 12 males per F_0 group, 42 days old, 175-204 gms
24 females per F_0 group, 81 days old, 198-257 gms

12 of the mated females in each group underwent Cesarean section on gestation day 20 and the remaining females were allowed to deliver.

Lactation day 0: 4M;4F pups/litter (if possible) were selected for evaluation of developmental indices and auditory and behavioral functions. Remaining pups were weighed on lactation day 4, examined externally, killed and discarded.

Remaining F_0 were sacrificed on lactation day 21. F_0 males were sacrificed after F_0 females had completed delivery.

F_1 Breeding: Cohabitation at a min. of 80 days of age with Cesarean section on gestation day 20 with delivery of F_2 generation. F_1 males were sacrificed and necropsied after final F_1 females C-section.

Results:

F_0 Generation: M;F survival 100% to sacrifice. High dosed females had an increased incidence of hair loss compared to controls. (Hair loss has also been seen in previous studies.) Male high dose had an increased incidence of soft stools. No other effects on appearance or behavior were noted.

Mid and high dose males showed slight but consistently reduced mean weekly body weights compared to controls from the first week of treatment on. During the treatment portion of study prior to mating, high dose female body weights were reduced compared to controls. Mid- and low dose females did not show similar findings. Treated groups mean body weight changes were comparable to controls throughout gestation and lactation.

Food intake for mid- and high dose F_0 males was slightly lower than that of controls during the premating period. The low dose group values were also slightly lower but with small differences. Food consumption for high dose F_0 females was lower than controls during treatment premating week 7. However, they were also lower during week 6 which was prior to dosing. Low and mid-dose females did not show similar findings. Although not statistically significant high dose rats ate less than controls during lactation.

Water consumption was generally decreased compared to controls during the first few weeks of study for mid- and high dose males. The low dose was comparable to controls. During the first week of treatment water consumption for high dose females was increased compared to controls. Reported that treated and control showed no meaningful differences during gestation and lactation.

However, though not statistically significant the high dose group consumed less water
than controls during lactation.

The frequency and type of estrous cycle abnormalities were reported as not biologically meaningful.

Cesarean section observations: Dams showed no apparent treatment-related findings at necropsy. Compared to controls there were slight to moderate decreases in mean numbers of implantations and viable fetuses per litter in the mid- and high dose levels with very slight increases in mean postimplantation loss/dam (control-high dose: 0.7, 0.9, 1.6, 1.3 - high dose statistically significant). Group mean postimplantation loss (%) = 4.6, 5.8, 11.9, 9.2%. High dose mean fetal body weights (3.3 gms) were very slightly reduced compared to controls (3.5 gms).

F_1 Fetal Morphological Observations: No malformations were seen in control or treated F_1 fetuses. The high dose contained a slight increase in the number of fetuses showing evidence of retarded skull ossification. The incidence of unossified Sternebra #5 was 23 and 23 for the low and high dose vs 12 and 12 for the control and mid-dose. Other variations were similar to controls.

F_0 Delivery and F_1 Litters: Reported that there were no biologically relevant differences in male and female fertility indices, copulatory rates or intervals, or lengths of gestation. Mean numbers of viable and nonviable pups of both sexes were statistically comparable to controls. There was a statistically significant decrease in the high dose pup survival index on lactation day 4 but not at later time periods (control-hd: 98.6, 98.2, 94.7, 61.9%) High dose mean pup body weights were statistically significantly lower than controls lactation day 0 through lactation day 21. Inhibition of body weight relative to controls continued at 28 and 35 days of age. Mean pup weights for the low and mid dose groups were reduced to a lesser degree, being significant on lactation days 4 and 7, and for the mid-dose on lactation day 0.

Appearance and behavior of pups that survived through lactation until 35 days of age were comparable with controls. 7, 3, 11, and 65 pups (control through high dose) died or were missing during lactation or before 28 days of age. 5, 2, 6 and 17 of these were necropsied and were normal internally (some were partially autolyzed). Additionally one mid- and 21 high dose were submitted for postmortem examination, however, extensive cannibalization precluded necropsy. Remaining missing pups were presumed completely cannibalized.

Disposition of F_0 Rats: At necropsy dams showed no treatment-related findings. No difference from controls was seen in mean numbers of implantation sites, delivered pups or remaining implantation sites.

F_1 Developmental and Behavioral Findings:

Static Righting Reflex: Comparable with controls lactation days 0-3.

Pina Detachment: In general comparable with controls although the percentage of pups with a positive response in the mid-dose was reduced on lactation day 2, but similar to controls thereafter.

Cliff Aversion: Positive on lactation day 11 except for one mid-dose pup which did not have a response until lactation day 12.

Eye Opening: No differences in bilateral eye opening from controls on lactation days 13-16; all had both eyes open by lactation day 16.

Air-Drop Righting Reflex: Positive - comparable with control lactation day 16.

Visual Placement: Positive response on lactation day 18.

Neuropharmacological Evaluation: All tested appeared normal lactation day 21.

Auditory Response: All tested gave a positive response at 33 days of age.

Rotorod Performance: At 34 days of age values for treated groups were slightly less than that for controls; the sponsor reports the differences were not great enough to be considered indicative of a treatment-related effect.

Activity and Emotionality: Values were comparable with controls.
Shuttle Avoidance: Compared to controls no adverse effects on learning or memory recall were noted in F_1 treated group rats of either sex.

F_1 General Observations:

All males and females selected for Shuttle Avoidance testing or breeding survived to scheduled sacrifice. Antemortem observations reported no biologically meaningful differences compared to controls.

High-dose mean body weights of F_1 males selected for breeding or behavioral evaluation were less than those of control from 6 weeks of age until sacrifice. Low and mid-dose F_1 males were also less than controls the first few weeks, but later were comparable with controls. Similarly females from the high dose group were consistently lower than controls from age 6 wks. until sacrifice. During the gestation interval (days 0 to 20) mean weight gain of the high dose was lower than that of controls. Low and mid-dose did not show this inhibition.

No apparent treatment-related findings were noted at C-section.

F_2 Fetal Morphological Findings: 1 low dose and 2 mid-dose fetuses had microphthalmia.

Necropsy Findings: No treatment-related findings were reported for the excess F_1 rats not selected for retention, the F_1 animals examined after Shuttle Avoidance testing, or F_1 rats examined after breeding.

Preliminary Teratogenicity Study of BTS 54 524 in Charles River CD Female Rats: . Report TX86053 dtd. Oct 86.
Batch: 1/R4, as P 24/59. Q.A.: Signed by QA Manager as monitored for GLP compliance (no inspection dates).

Study conducted for determination of dose levels for main study (below).

Phase 1: Groups of 3, 3, 4 and 3 pregnant rats received 0, 3, 10 or 30 mg/kg daily by oral gavage (1 ml/100 g) from day 6-16 postcoitum (housed on sawdust in solid-bottom cages).

One high dose was killed on day 10 and another on day 13 because they had developed ventral body lesions. Both had shown signs of CNS stimulation and stereotyped behavior, had lost weight, and had reduced food intake. They appeared to have ingested sawdust.

Subsequently groups of 5 pregnant rats received 20 or 30 mg/kg daily from day 6-16 p.c. [housed on raker-bottom (R) cages]. Sacrifice was on Day 21 p.c. One 20-R mg/kg rat was killed day 9 and another on day 18; 3 30-R mg/kg were killed day 8 - all developed lesions, mainly on paws. Findings were similar to that of the first group; no significant findings were reported at necropsy.

10 mg/kg and higher produced behavioral changes; sparse hair areas were seen in some of all treated groups. Overall mean bodyweight increases were lower for treated and generally paralleled reduced food intake. There was a higher incidence of staining on the face and head and piloerection.

Neither embryonic nor fetal toxicity was evident. There was no dose-response relationship, but the incidence of fetuses with abnormalities and the percent with supernumerary ribs was slightly higher in some treated groups.

It is reported that findings were quantitatively and, with respect to some behavioral changes, qualitatively different from those in a 14-day study in non-pregnant rats. Behavioral changes were seen in both studies, but there was no stereotypy and no lesions in the 14-day study and effects on bodyweight and food consumption were less marked.

Thus Phase 2 was conducted with mated and unmated rats.

Phase 2: Groups of 5 pregnant rats received 5, 10, 20 mg/kg/daily, and groups of 4 pregnant rats received 0 or 15 mg/kg/daily by oral gavage from day 6 to 16 postcoitum. In addition groups of 5 unmated female rats received 0,

5, 10, 15, 20 mg/kg daily by oral gavage day 6-16 of study (housed in raker-bottom cages). Pregnant killed and examined Day 21; unmated killed on Day 21 but not examined.

One 20 mg/kg rat was killed day 8 p.c. because of paw lesions. Mated and unmated showed behavioral changes, overt signs and effects on bodyweight and food consumption consistent with Phase 1.

The mean number of live fetuses in the 20 mg/kg dose group was slightly less than that of controls; there was a corresponding slight reduction in fetal viability and a small increase in the number of resorption sites. At 5, 10 and 15 mg/kg day the mean fetal weight was slightly lower than that of controls; there was a corresponding retardation of skeletal calcification.

The incidence of abnormalities was slightly higher for treated than for controls, however, no dose-response was reported and no findings consistent with Phase 1. One each 5 and 20 mg/kg had multiple abnormalities, the one from the 5 mg/kg group and another from this group and 2 of the 15 mg/kg group had head defects. No comparable defects were seen in controls. Two 5 mg/kg fetuses had wavy ribs. The proportion of fetuses with findings found at external, visceral or skeletal examinations was slightly higher for treated than controls [control through hd. = 1/58 (2%), 5/72 (7%), 3/68 (4%), 3/56 (5%) and 6/49 (12%) and the number of litters were 1/4, 5/5, 2/5, 1/4, and 2/4.

Unmated - On one day one 15 mg/kg unmated animal had clonic convulsions when handled.

The reproductive status of the rats appeared to be of no consequence for toxicological findings.

Results of the two phases were not consistent. Phase 2 showed evidence of embryotoxicity at 20 mg/kg (highest dose) and fetotoxicity and retarded calcification at 5, 10, and 15 mg/kg - there were no similar effects at comparable dosages in phase 1. Small numbers also preclude relevance of the findings.

The sponsor determined the suitable highest dosage for the main study would be 10 mg/kg.

Teratogenicity Study of BTS 54 524 in Charles River COBS CD Female Rats: Report

TX86123, 510-011 dtd. Nov, Dec 1986. Batch: 2 as p21/37
Q.A.: Present. Study according to Japanese Guidelines.

Dose: 0, 1, 3, 10 mg/kg/day orally by gavage as a single daily dose to F_0 days 7 through 17 of gestation. Vol. 10 ml/kg/day
Vehicle deionized water.

No. Animals: 40 females per group Age ca 15 wks. 220-323 gms

Cesarean section 20 females per group on gestation day 20. Remaining 20 females (20/gp) were allowed to deliver. F_1 pups were selected from each litter and evaluated for behavioral and developmental indices (Shuttle Avoidance Test). F_1 M;F were selected from each litter and mated to assess reproductive capabilities. All F_1 dams were sacrificed for C-section on gestation day 20.

Results:

The only effects on dams were retarded weight gain and inhibited food intake during treatment at all dosages and hair loss from limbs and ventral body surface in some high dose rats.

It is reported that assessment of primary markers of developmental toxicity revealed no evidence of an effect on the offspring and further at levels up to 10 mg/kg there were no increases in fetal morphological aberrations indicative of teratogenicity or increases in postimplantation loss indicating fetal death. Growth both in utero and postpartum was comparable with controls.

No indication of functional disturbance was indicated for F_1 offspring from the treated groups from developmental and behavioral values and reproductive performance.

F_0 Maternal Observations: All F_0 survived to terminal sacrifice.

Most high dose lost weight after the first dose, after which they gained weight, but the mean weight change over the treatment period was less than controls. Effects in the low and mid-dose groups were similar but less marked. During pregnancy overall weight gain was lower for all treated groups. From lactation days 0-21, mid and high dose mean maternal weight gains were greater than that of controls. The low dose was comparable to controls.

Food intake values of treated groups showed a dose-related decrease relative to controls on gestation days 7-14 only. There was no consistent effect on water intake.

At C-section there were no apparent differences in mean numbers of viable fetuses, postimplantation loss, total implantations, corpora lutea or fetal sex ratios. The mid-dose (but not the high dose) had a very slightly but significantly lower mean fetal body weight. Mid and high dose mean placental weights were very slightly (but significantly) higher than that of the control group.

F_1 Fetal Morphological Findings:

Malformations were present in one fetus each in the control, low and mid-dose groups and in 5 fetuses from 4 high dose litters. Defects were not the same in the 4 litters - i.e. 1 Control - ablepharia, microstomia, mandibular micrognathia; 1 low dose - diaphragmatic hernia; 1 mid dose - microphthalmia; 5 high dose - 2 with hydrocephaly, 2 with thread-like tail with small anal opening, 1 with misshaped, fused and maligned vertebral arches.

Both the incidence and type of developmental variations were similar to that of controls. Although not statistically significant the numbers of fetuses with #5 and #6 unossified sternebrae were slightly increased in the mid and high dose.

F_0 Delivery and Litter Findings:

One pregnant control failed to deliver and was killed on day 25 - uterine examination showed two implantation sites. Another control delivered only 1 pup which died on lactation day 1. No effects were reported on gestation length, the number of viable and non-viable at birth, pup survival and body weight during the lactation period. Survival and body weights of weanlings up to 35 days of age were not affected.

Appearance of most pups during the lactation period was normal. A number of treated pups were born dead or died during the lactation period as follows 4, 10, 10, 19 (control - high dose). 3, 4, 6, and 12 of these were necropsied; all appeared normal or had normal autolytic changes. Remaining pups were either partially or totally (missing) cannibalized.

At necropsy dams showed no apparent treatment-related findings. There was no difference between groups in the number of implantation sites which did not correspond to delivered pups.

F_1 Developmental and Behavioral Findings:

Static righting Reflex: Comparable to controls; all tested pups had a positive response by lactation (l) day 5.
Pinna Detachment: Comparable to controls.
Cliff Aversion: Positive on lactation day 11.
Eye Opening: Comparable with controls - both eyes open by lactation day 16.
Air-Drop Righting Reflex: Treated and controls positive on l-day 16.
Visual Placement: Treated and controls positive on l-day 18.
Neuropharmacological Evaluation: Normal on l-day 21.
Auditory Response: Control and treated positive at 33 days of age.
Rotorod Performance: Day 34 - Positive response slightly lower for treated. Values were not dose related and close to those seen in previous controls.
Activity and Emotionality Findings: Not meaningfully different from controls.
Shuttle Avoidance Findings: No apparent effects of treatment.

F_1 General Observations:

One low dose male selected for breeding died at 14 weeks of age. This animal had a small liver but the cause of death was not determined. Antemortem observations were similar for all groups.

F_1 male and female mean weekly body weights were similar for all groups, and mean maternal body weight gains during gestation were also similar.

Cesarean section showed that treatment did not affect fetuses or litter parameters.

F_1 Necropsy Observations: Reported - No apparent treatment-related findings. However, findings included the following:

	F_1 Sacrifice Control through High dose	
Kidney		
Hydronephrosis		
Scheduled 35 days of age:	Male (0, 1, 0, 2)	Female (0, 1, 1, 4)
Shuttle group age 14-15 wks:	Male (0, 0, 1, 0)	Female (0, 1, 0, 3)
Breeding group age 15-17 wks:	Male (0, 0, 2, 1)	Female (0, 0, 1, 4)
Lung		
White foci		
Scheduled 35 days of age:	Male (none)	Female (none)
Shuttle group age 14-15 wks	Male (0, 3, 0, 3)	Female (1, 0, 1, 2)
Breeding group age 15-17 wks:	Male (0, 0, 0, 1)	Female (1, 1, 0, 1)
Cysts		
Scheduled 35 days of age:	Male (none)	Female (none)
Shuttle group age 14-15 wks:	Male (0, 0, 4, 4)	Female (4, 3, 1, 2)
Breeding group age 15-17 wks:	Male (none)	Female (1, 0, 0, 0)

F_2 Fetal Morphological Findings: One low dose had ectrodactyly and another low-dose had omphalocele.

Preliminary Oral (Dose Finding) Study of BTS 54 524 in Non-Pregnant Female SPF Dutch Belted Rabbits: Report TX86055 dtd Oct 86. Batch: 2, as P21/37.
Q.A.: Signed QA Manager as incompliance with FDA GLPs (no inspection dates).

This study was conducted in two phases to determine the dose levels to be used in a preliminary teratogenicity study.

Phase 1. One pair of female rabbits received single oral doses of 3, 20 and 90 mg/kg on days 1, 3 and 8 of the study, respectively, and another pair received 10, 40 and 100 mg/kg on days 2, 7 and 9, respectively; a third pair was given vehicle, purified water B.P. as a control.

One 100 mg/kg rabbit was killed the day it received the 100 mg/kg dose because of a neck lesion. It had dilated pupils, rapid respiration, stereotyped behavior and increased sensitivity to noise and movements of the handler. All 40, 80 or 100 mg/kg rabbits showed dilated pupils, rapid respiration, and increased sensitivity to noise. Rabbits given the two higher doses had stereotyped behavior and made abnormal head movements on the dose day. 80 mg/kg rabbits and the 100 mg/kg survivor lost weight the day after dosing. Postmortem examination showed no abnormal findings.

Phase 2. Pairs of female rabbits were given oral doses of 25, 50, 75 mg/kg for 13 consecutive days then killed day 14 and examined. Findings including weight loss were similar to those above the two higher doses being affected more often.

The sponsor concluded that 75 mg/kg would be a suitable highest dosage for the preliminary teratogenicity study in rabbits.

Preliminary Teratogenicity Study of BTS 54 524 in Female SPF Dutch Belted Rabbits: Report TX86068 dtd Oct 86. Batch: 2 as P21/37. Q.A.: Signed QA Manager as incompliance with FDA GLPs (no inspection dates).

Study to determine dose levels for teratology study.

Groups of three, four, five, five and four pregnant rabbits (5/group mated) received 0, 5, 10, 50, 75 mg/kg daily from day 7 to day 19 postcoitum.

Rabbits given 10 mg/kg daily or higher showed behavioral changes comprising evidence of CNS stimulation and stereotyped behavior and overt signs comprising dilated pupils and rapid respiration. Some animals mainly in the higher dosages lost hair and had lesions probably as a consequence of excessive grooming. The two higher doses lost weight during dosing but began to regain weight ca day 15 and 19, respectively. None of the treated groups gained as much weight as controls. One 10 mg/kg rabbit lost weight throughout the study. One animal each from the 50 and 75 mg/kg groups had an erosion or red foci in the stomach which may have been treatment related.

Litter parameters were not affected. However, the 10 and 75 mg/kg groups showed a slightly higher proportion of fetuses with one or a pair of additional ribs. Although this may be a common finding, the influence of treatment cannot be totally excluded. The incidence of non-calcified forepaw elements was slightly increased in the 50 mg/kg group.

The sponsor concluded that 75 mg/kg would be appropriate for the highest dosage of the main teratology study.

Teratogenicity Study of BTS 54 524 in Dutch Belted Rabbits: Report TX88093 Code LR 0016 dtd. Oct 88. Batch: No. 2 as P21/37 Q.A.: Present (Amend 1,5 Aug 96).

Dose: 0, 3, 15, 75 mg/kg daily from day 7 to day 19 postcoitum by oral gavage. Solutions in Purified Water BP at conc. of 0.75, 3.75 and 18.75 mg/ml. Vol 4 ml/kg.

No. Animals: 14, 15, 14, 16 pregnant SPF Dutch Belted rabbits (16 per group were mated)

Sacrifice was on Day 30 postcoitum.

Although 75 mg/kg daily seemed to be a satisfactory highest level, this dosage proved too toxic in the main study - it was not tolerated by a sufficiently high proportion of the dams and high dose animals had a higher incidence of gastric lesions.

Results:

Marked CNS stimulatory effects and stereotypy were evident which were generally normal 24 hrs. after dosing. Such effects were largely responsible for the death of 1 dam after the second dose. This animal damaged its teeth due to stereotyped gnawing behavior. There were blood clots and prominent blood vessels in the brain, the upper incisors were damaged and there were 2 erosions in the stomach. Nine implantations were of the correct size for day 8.

The 15 mg/kg dose was better tolerated than the high dose - dams however, showed CNS stimulatory behavior. Effects at 3 mg/kg consisted of one rabbit with occasional stereotypy.

All high dose rabbits lost weight during treatment; 3 failed to recover even after the end of the dosing period and were killed moribund (2 on day 21 and 1 on day 23). These rabbits had shown stereotyped behavior. One killed on day 21 also had dilated pupils, rapid respiration, hair loss and a reddish brown exudate from the vagina, pale and mottled kidneys, pale liver with prominent vessels, and many black foci in the stomach. Uterine horns contained bloody fluid and 9 dead fetuses. The other Day-21 animal had pale lungs, a pale area on one kidney, and pale raised areas in the stomach which had dark contents. The uterus had 7 dead fetuses, 5 which were of normal size and 2 which were macerated. The rabbit killed on day 23 had prominent blood vessels on the surface of the heart, the periphery of the right atrium was pale, cut kidney surfaces were dark with pale areas on the papillae, the liver was pale and had a small red area on the margin of one lobe, stomach contents were dark, GI contents were gaseous, the left oviduct was displaced, the left uterine horn had reddened walls and contained opaque cream material, and there was blood in the lumen of the right uterine horn. The uterus contained 4 dead fetuses, 3 were normal and correct size and the remaining one was too macerated to examine.

Hair was found in the stomachs of these rabbits (excessive grooming!).

In addition one high dose and one control started littering on day 30 p.c. The control delivered 2 normal live young - at autopsy four live fetuses and one dead one were found in the uterus. The high dose rabbit appeared thin and cool and breathed slowly. It delivered 6 dead fetuses (four of which had recently died) - at autopsy another dead fetus was found in the vagina.

Hair loss, more prominent at the high dose and probably a consequence of excessive grooming, sometimes led to skin lesions developing on the limbs, axillae, abdomen and tail. Most high dose rabbits and 3 from the mid-dose group voided fewer feces, predominantly during the dosing period, and some of these appeared to drink less water.

Weight loss was generally seen with the 75 mg/kg dose throughout the dosing period ($p < 0.01$). After dosing was discontinued, most began to regain weight, and by the end of study most weights were similar to, or slightly greater than, controls. 15 mg/kg animals generally lost some weight after the first dose after which changes in bodyweight were similar to controls.

Post mortem examination of the dams showed discolored areas and erosions in the stomach of one control, 3 low dose, 1 mid-dose and 4 high dose.

At 75 mg/kg there was a slightly reduced number of live fetuses per litter with a correspondingly slight increase in the incidence of intra-uterine death (dead fetuses plus resorption sites). At 15 mg/kg the mean number of intra-uterine deaths was slightly but non significantly increased. Fetal viability for the high dose was significantly lower than for controls ($p < 0.01$). The very slight decrease for the 15 mg/kg group was not significant.

Fetal weight appeared to be slightly reduced (non sig) at 75 mg/kg which correlated with a slight reduction in the extent of fetal skeleton calcification.

The sex ratio was widely variable without apparent treatment related differences.

There were no inter-group differences in the numbers of dams with abnormal fetuses or in the numbers of abnormal fetuses. However, there was a possible slight increase in high dose fetuses with the syndrome consisting of a broad short snout, short rounded pinnae and short tail with multiple caudal vertebral anomalies. Some of these fetuses also had thickened shorter long bones of fore and hind limbs. The incidence of fetuses was 4/83, 2/85, 0/78 and 8/56 control through high dose, and 3, 1, 0 and 2 for the number of litters.

[Reported that this syndrome has been found in rabbits from this source maintained in other laboratories and its origin has been traced to a small number of bucks. The six affected dams in the present study were mated by four bucks, one of which also sired a litter containing this syndrome which was not part of the present study. Thus, according to the sponsor this syndrome appears to be mediated through the male (not the treated female). Although there were no such defects in the preliminary study, marked maternal toxicity at the

highest dose could have exacerbated the situation and resulted in a slightly higher incidence of abnormal fetuses.]

In addition, some fetuses in all groups showed retarded calcification, variously involving the sternebrae, vertebrae, hyoids, pubes, metacarpals, tarsals and phalanges. The incidence was higher for the high dose group with the numbers of affected fetuses being 12/83 (14.5%), 4/85 (4.7%), 16/78 (20.5%) and 21/56 (37.5%), control through high dose.

Background Study of BTS 54 524 in Pregnant SPF Dutch Belted Rabbits:
Report TX90081 Code LR 0017 Dec 1990.
Batch: (?) Q.A.: Reported as Not available (Amend 1,5 Aug 96).

The teratogenicity study (TX88093) conducted in SPF Dutch Belted rabbits given sibutramine showed an increased incidence of a syndrome of malformations (short and rounded pinnae, short and broad snout and short tail) in fetuses from the high-dose (75 mg/kg) dams when compared to controls. The following study was conducted to provide more information that this syndrome can occur spontaneously in this strain of rabbit and ensure to be mediated through the

mated female rabbits were allocated to each of 4 groups (A, B, C, and
that only one in each group had been mated with each of the 14 stud
o preclude biasing any group by the use of possible "carrier" bucks).
tigate the possibility of dosing stress influence on pregnancy, rabbits
s A and B were given Purified Water BP (4 ml/kg) orally by gavage from
o 19 post coitum and Groups C and D were not dosed. Animals were
ed on day 30 postcoitum.
1, 1, and 0 rabbits in groups A, B, C and D died or aborted and were
[Prior to start of study 9/72 stock females originally supplied for
ed or had to be killed due to poor condition. Animals that died or
included one Group A with 10 dead fetuses, one that aborted five and
that aborted 8 dead fetuses, a Group B that aborted 5 dead fetuses and
C that aborted 7 dead fetuses. In addition 2/72 of the females
ly supplied had the affected syndrome. It is also now reported that the
was also manifest in an adult female that was supplied for the
ine study.] Treated animals had shown marked bodyweight loss prior
, but there was no obvious cause of death. The only difference between
hat was attributable to dosing was some superficial damage to the
mucosa of the dams possibly caused by the catheter tip. Fetuses with
findings (external, visceral or skeletal were 8/48, 8/54, 8/53 and
r Groups A-D. The incidence of more than 12 pairs of ribs was 39/48,
0/53 and 28/47.
most significant finding in this study was the appearance of the
of birth defects in five fetuses from 2 group B (water) litters. The
believes this to confirm that the syndrome of defects that occurred in
tramine study was not specifically caused by the compound. [The
is still being expressed in these rabbits and thus they are considered
sponsor as presently not suitable for evaluating the teratogenic
l of BTS compounds.]

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ary (Teratology) Study of Sibutramine Hydrochloride in New Zealand White
Report TX91030 Code LR
April 1991. Batch: no. 4 Q.A.: Present - In compliance with
Health UK Compliance Programme.

e: 0, 3, 15, 50, 75 mg/kg from day 7 to day 19 post coitum by oral gavage at a vol. of 4 ml/kg

Prelimina
Rabbits:
0018 dtd.
Dept. of

Dose

No. Animals: 4, 4, 6, 6, and 5 pregnant animals

75 mg/kg proved to be too toxic and the animals were killed after a few doses. Behavioral changes, overt signs and bodyweight losses were proportionately greater than that seen in the Dutch Belted rabbits.

CNS stimulation appeared in the 15 mg/kg rabbits and more frequently in the 50 mg/kg group where stereotyped behavior was evident.

After the first dose all 50 mg/kg animals (and some 15 mg/kg which recovered quickly) lost weight until day 11 p.c. Bodyweight gain was similar to controls after the initial response.

There were no apparent treatment-related findings in dams at post mortem examination.

The 50 mg/kg group had a slightly higher mean number of intrauterine deaths, and fetal viability was slightly lower than controls; also the mean bodyweight of female fetuses was lower than controls.

The incidence of fetal abnormalities showed no treatment-related effects; the incidence in the high dose group (11/47) was less than that of controls (16/38). All litters showed fetal variants and the incidence of fetuses with minor differences in pelvic girdle shift (a variant in which the pelvic girdle is inserted caudally to the first sacral vertebra) appeared to be slightly higher in the 50 mg/kg group. Asymmetrical insertion of the pelvic girdles was seen in 3/42 at 3 mg/kg, 7/43 at 15 mg/kg and 2/47 at 50 mg/kg vs none in controls.

The 3 mg/kg group showed no adverse effects.

50 mg/kg was concluded to be the suitable high dosage for the main study.

Oral Teratogenicity Study of Sibutramine Hydrochloride in New Zealand White Rabbits: Report TX92015 Code LR 0019 dtd March 1992. Batch: 4 as W/O 44594/RM Q.A.: Present.

Dose: 0, 3, 12, 50 mg/kg day 7 to 19 postpartum by oral gavage at a vol. of 4 ml/kg.

No. Animals: 15, 14, 15, 13 pregnant New Zealand White Rabbits.

Sacrifice was on Day 30.

Signs of CNS stimulation and stereotyped behavior comprising excessive chewing, sniffing and grooming, nodding head and gnawing or licking the cage were seen in the 50 mg/kg group from ca. 30 min to 6 hrs. 12 mg/kg occasionally produced dilated pupils. At 3 mg/kg there were no overt or treatment-related signs. Two 50 mg/kg rabbits were replaced after administration of the first dose, one which convulsed and died and one with weak limbs and slow reflexes. One low dose rabbit aborted one dead fetus and was killed day 20; 12 dead fetuses remaining in utero were too small to examine. The dam showed no effect on bodyweight, no overt signs of toxicity and no apparent treatment-related findings. Another low dose dam aborted 1 dead fetus (12 remaining fetuses in utero were too small and macerated to examine) and was killed on day 23. This dam had shown marked weight loss two days prior to abortion and a pinched body, cool to the touch, hunched and piloerect and a red exudate from the vagina. The liver had cream mottled areas.

At the start of the dosing period the 50 mg/kg dams lost more weight than the other groups after which bodyweight gain for all groups was similar. Over the complete dosing period mean weight gain for the 50 mg/kg group was lower (mean bodyweight gain 59% of control) and for the 12 and 3 mg/kg groups slightly lower than controls. Dams from all treated groups generally gained more weight than controls after the end of the dosing period.

Post-mortem examination of the dams showed no consistent treatment-related findings. The incidence of reddened ovaries was greater than controls for the two higher dose groups.

The mean number of corpora lutea showed little differences between the groups. The lowest implantation index was seen at 3 mg/kg and the mean number of resorption sites and live and dead fetuses were not adversely affected by treatment. Treatment did not adversely affect viability and the widely variable sex ratio showed no apparent treatment-related differences.

Mean fetal weight was lower in the 50 mg/kg group (consistent with increased mean litter size) than in other groups. Each of 5 treated dams had one fetus (1, 2 and 2 from the 3, 12, 50 mg/kg groups) with cardiac anomalies. Also one fetus from a dam (maternal tissues normal) that had received a single 50 mg/kg dose had an abnormal heart. There were no cardiac anomalies in concurrent controls. [According to the sponsor, although atypical in the present study the incidence in treated groups is consistent with the background incidence in this strain of rabbits and considered to be of genetic origin.] 4/5 of the abnormal hearts showed similar morphological changes i.e., stenosis or atresia of the pulmonary trunk and/or stenosis of the right atrio-ventricular valve. The other heart showed slight enlargement of the aortic arch and the left ventricle with slight reduction of the right ventricle. Internally, there was a septal defect between the exit of the aorta and the right ventricle. [The percent of control fetuses with stenosis or atresia of pulmonary trunk or valve from 4 different laboratories varied from 0-0.8%. The overall frequency (i.e. all four groups and dam taken off after single 50 mg/kg dose) in this study was 1.2% (5/415).]

Treated groups had an increased incidence of two variants, thirteenth rib and displacement of the pelvic girdle [pelvic girdle shift in which the pelvic girdle was inserted on the second or first and second vertebrae]. This finding was greatest in the 50 mg/kg group (51%) and 3 mg/kg (17%) compared to the control (27%). According to the sponsor, this is a developmental variant and has no effect on health or body conformity and represents only a slight deviation from normal.

The sponsor concluded that sibutramine hydrochloride was not teratogenic in New Zealand White rabbits at doses up to 50 mg/kg daily from days 7 to 19 post coitum although maternal toxicity was elicited.

Teratogenicity Study - Effect of Sibutramine on Pregnancy of the New Zealand White Rabbit: Report TX94043 BTS 271/931004 March 1994. Batch: No. 5 Q.A.: Present.

Dose: 0, 12, 24 mg/kg Days 6 to 18 post coitum by gastric intubation. Given in distilled water at 4 ml/kg.

No. Animals: 32-33 time-mated female rabbits 16-24 wks of age 3.15-4.00 kg.

Sacrifice on Day 29.

Two satellite groups at 12 and 24 mg/kg were included to provide blood samples on Days 6 and 18 p.c. (first and last days of dosing) at 0, 1, 2, 7, 12, 24 hrs. after dosing for analysis of plasma levels of sibutramine and metabolites. Pregnancy status of these animals was confirmed on Day 19 but they were not examined further - thus individual parameters were reported but they were not included in group value tables. (Results of plasma analysis are not included in this HRC report.)

Maternal observations: There were 4 mortalities: 1 control, 1 low dose and 2 high dose. The sponsor did not consider these deaths to be treatment-related.

Dose related increases were seen in frequency of pupil dilation (occurred only during the dosing period) and in more frequent occurrences of cold ears (a general stress response of rabbits). Throughout the dosing period the 24 mg/kg dose had a more frequent occurrence of low fecal output.

There was a dose-related initial mean bodyweight loss Days 6 to 7 of pregnancy. For the two higher dosages the loss continued Day 8 for the 12 mg/kg group and Day 9 for the 24 mg/kg group (statistically significant, $p<0.05$). After this bodyweight gain was similar for all groups, however, the high dose did not attain parity with control by termination.

There was a dose-related reduction in mean food consumption during the first 3 days of treatment. The 12 mg/kg group was essentially similar to that of controls after Day 8. The 24 mg/kg group made a slight recovery but remained less than that of controls throughout treatment. Statistical significance was attained Days 6-12. Both dosages were similar or superior to that of controls from the end of treatment to termination.

Litter and fetal observations: Corpora lutea, live young, sex ratio, litter weight, mean fetal weight and sternal variations (ossifications) showed no apparent treatment-related adverse effects. In addition with respect to the incidence of embryonic deaths, and malformations in general no adverse effects of treatment were evident.

However, visceral and skeletal anomalies were significantly increased at 24 mg/kg. There were statistically significant ($P<0.05$) increases in the incidence of litters/fetuses with deviations in the origin of small arteries from the aorta and in the incidence of litters/fetuses with ossified connection between the jugal and maxilla. The high dose also had a significantly higher incidence of fetuses with 13 ribs. At 12 mg/kg such differences were marginal and not statistically significant.

The sponsor made no specific conclusions regarding teratogenicity.

Perinatal and Lactation Study of BTS 54 524 in Charles River Crl:CD VAF/Plus Rats: Report TX91082 dtd. Oct 91. F_0 mating initiated Aug 1989. Batches: 4.QC MO. 00470H; 4.QC00470H Q.A.: Present.

Dose: 0, 1.0, 3.0, 10.0* mg/kg orally by gavage gestation day 17 through day 21 of lactation. Controls deionized water. Vol 10 ml/kg.

Due to excessive toxicity at the high dose level, an additional treatment group receiving a dosage level of 0.1 mg/kg/day and an additional concurrent control group were added after the sacrifice of the F_0 females in the original control and treated groups. Delivered females were inadvertently not dosed on the day of sacrifice, lactation day 21.

*10 mg/kg group - dosing terminated late in gestation or early in lactation (gestation day 19, 20, 21, 22 or lactation day 0).

No. Animals: 25 rats per group ca 13 weeks old, weight ca 211 and 196 gms. Additional groups ca. 11 wks of age and ca. 204-254 gms.

F_0 females were allowed to deliver. F_1 pups were selected from each litter (8 pups of equal sex distribution if possible) and evaluated for behavioral and developmental indices. To assess reproductive capabilities selected F_1 males and females from the same treatment groups were mated to assess their reproductive capabilities.

On gestation day 20, C-sections were performed on the F_1 females and F_2 fetuses were examined externally for teratological findings.

Due to early termination of 10 mg/kg F_0 females, no F_1 adult or F_2 fetal data were available for this group.

Litters were caged with their dams for 3 wks following birth. Any intact pups found dead were necropsied. The heart was dissected and the carcasses discarded.

Pups were separated by sex on lactation day 21. At ca 35 days of age they were housed individually and 25 males and 25 females per group (with a minimum of 1M;1F from ea litter) were randomly selected for F_1 mating.

Results: [Comparisons to concurrent controls.]

One 10 mg/kg female died on gestation day 21 and one on lactation day 0. For the first dam, no delivered pups were seen, stated presumably due to cannibalism, based on empty implantation sites noted at necropsy, delivery had initiated. There were however, no signs of dystocia and no agonal antemortem findings. The lactation day 0 dam was pale, ataxic, emaciated and gasping with black nasal discharge prior to death. No gross lesions were evident at necropsy for either female and the cause of death could not be determined. Survival was 100% in the remaining groups.

At the 10 mg/kg level, maternal F_0 toxicity included mortality, reduction in body weights and food and water consumption and abnormal behavior which included aggressiveness, hyperactivity, increased sensitivity to touch, and cannibalization of apparently healthy offspring. Such behavior in the majority of the females contributed to a marked reduction in survival of F_1 offspring at this level. Approximately half of the dams that littered were seen chewing on their forelimbs, along with scabbed areas on the forelimbs and focal hair loss. During this interval a few high dose females showed increased activity, high carriage and red material (presumably blood) around an eye or the anogenital region.

The 3.0 mg/kg F_0 females had increased hair loss, but did not have the abnormal behavior, including chewing the forelimbs, nor scabbed forelimbs.

The 1.0 mg/kg and 0.1 mg/kg groups were comparable to concurrent controls.

Treatment-related toxicity at the 1 and 3 mg/kg levels included reduced F_0 maternal weight gain (sig.), food and water consumption during treatment period of gestation (Days 17-20). Body weights were decreased for the F_1 as neonates and males as adults. During the lactation period the 0.1, 1.0 and 3.0 mg/kg mean maternal body weights or body weight changes were similar to controls.

The 0.1 mg/kg level produced a reduction in F_0 maternal weight gain during gestation day 20 which was transient (days 14-21) and not accompanied by reduction in food or water consumption.

At lactation day 0 the mean body weight of 10 mg/kg F_0 dams was significantly reduced compared to controls.

During lactation, food and water consumption of the 3.0 mg/kg group was reduced. Slight reductions were also seen at 0.1 mg/kg but not at 1.0 mg/kg.

F_0 Reproductive and F_1 litter Parameters: The 10 mg/kg group showed a statistically significant reduction in the gestation index. Cannibalizing of apparently healthy offspring occurred in the majority of dams of this group. Decreased survival of F_1 offspring was also observed at 3.0 mg/kg; maternal cannibalism was seen for some of the offspring in 5/6 litters with 100% offspring mortality. One of these litters also had maternal neglect. Control and treated mean gestation lengths were comparable.

Offspring Survival: At 10 mg/kg there was a marked increase in the mean number of stillborn offspring and statistically significant reductions in mean numbers of live males and females at lactation day 0. In this group the majority of offspring were dead or sacrificed by lactation day 4 due in part to maternal cannibalism. The majority of offspring in the remaining four litters sacrificed for ethical reasons on lactation day 2 or 3 were alive and appeared normal.

At 3 mg/kg the highest mortality occurred by day 4 although there were statistically significant reductions at days 0, 7 and 14. Six litters in this group had 100% mortality. At lactation day 0 the mean number of live males was reduced compared to controls. However the mean numbers of live females and dead offspring at lactation day 0 were comparable to control.

0.1 and 1.0 mg/kg groups showed no significant differences in survival indices. At lactation day 0 the mean number of live females (but not males) was reduced. There was no increase in the mean number of live stillborn and no treatment-related differences in sex ratio at lactation day 0.

Offspring Growth: At 10 mg/kg significant decreases in mean body weight were noted at lactation day 0.

The 1.0 mg/kg group and 3.0 mg/kg females were comparable with controls. Males of the 3 mg/kg group showed a slight but statistically significant weight decrease on lactation day 0. Dose related decreases occurred days 4-35 some of which were significant. 0.1 mg/kg showed no treatment-related effects.

Offspring Assessment: 10 mg/kg pups appeared small in size. The dead and sacrificed that were available appeared normal internally.

Small size also appeared with an increased incidence at 3.0 mg/kg. A few control and/or treated showed sparse hair coat, paleness, lacerations, reduced motor activity, gasping, discolored limb or tail and/or missing distal tail. One control pup had anophthalmia; another control had an abnormal gait and dome-shaped head (hydrocephaly).

2, 2, 1 and 13 (orig. control, add. control, 1, 3 mg/kg groups) were missing and presumed cannibalized and an additional 6 offspring in the 3 mg/kg group were too cannibalized to examine - in this group 2 partially cannibalized were normal where intact.

Internally the majority of F_1 offspring were normal at necropsy, however some dead pups had autolysis. One 3.0 mg/kg pup had stenosis of the aortic arch. 1.0 mg/kg malformations included situs inversus in one weanling and interventricular septal defect in two littermates. The second control group had two littermates both with kidney and ureter agenesis. Findings with no dose-related instance in control or treated included hydronephrosis, distended ureter, uterine horn reduced to a ligament and hemorrhagic testis.

F_0 Uterine Observations at Weaning: At 10 mg/kg there was a reduction in the mean number of delivered offspring with an increase in implantation sites which did not correspond to those delivered; there appeared to be postimplantation loss and/or complete cannibalization of the offspring relative to controls. The mean number of implantation sites was comparable to its original control group.

The 1.0 and 3.0 mg/kg groups showed no treatment-related differences in the mean number of delivered pups, the incidence of postimplantation loss/complete cannibalization or the mean number of implantation sites.

Compared to controls the 0.1 mg/kg group showed reductions in the mean number of implantations (however, treatment began several days after implantation) and in the mean number of offspring.

F_1 Behavioral and Developmental Indices: [Offspring from the 10 mg/kg group were evaluated only for static righting reflex and pinna detachment due to early group termination.] The following indices were within normal or comparable to corresponding control values: Static Righting reflex, Pinna Detachment, Cliff Aversion, Eye Opening, Air Drop Righting Reflex, Neuropharmacological Observations, Auditory Response, Rotarod Performance, Activity and Emotionality, and Learning and Memory Testing-Passive Avoidance.

F_1 General Observations:

Survival, Appearance and Behavior - one 0.1 mg/kg/female died at 9 weeks of age - cause unknown.

F_1 adult rats showed no apparent treatment-related differences in appearance, behavior or incidence of necropsy findings.

Body Weights - 1.0 and 3.0 (but not 0.1 mg/kg) F_1 males showed dose related reductions in mean weekly body weights compared to original controls weeks 6 - 20. F_1 females were comparable to controls.

Reproductive Parameters - For F_1 treated groups male and female fertility indices, copulatory indices and mean copulatory interval were comparable to controls. Sperm were not motile for one control group that failed to sire, but were present, motile and morphologically normal in all other F_1 males that underwent spermatogenesis testing. Control and treated groups showed little difference in mean absolute or relative testis or epididymis weights of F_1 males that failed to sire.

Cesarean Section Observations - Compared to controls there were statistically significant increases in mean fetal body weights in treated groups (low and high dose 3.5 and 3.7g vs 3.4g for controls). The mean number of viable fetuses, postimplantation loss, implantations, corpora lutea, placental weights or fetal sex distribution showed no treatment-related or statistically significant differences.

F_2 Fetal External Morphological Observations:

Malformations - No external malformations were observed.

Developmental Variations - No dose-related differences were noted in external developmental variations.

According to the sponsor, the oral no observable effect level was 0.1 mg/kg, 1.0 mg/kg for F_1 behavior and developmental parameters (reduced survival of 3.0 mg/kg F_1 offspring) and 3.0 mg/kg for F_1 reproductive capacity.

Perinatal and Lactation Study in Mated Female Charles River Crl:CD VAF/Plus Rats Utilizing Cross-Fostering:

Report TX91011 dtd Feb 91. Batch: 4 QC 00470H, Kek milled as w/o 44594/RM Q.A.: Present.

This study was conducted to determine and characterize the possible adverse effects of BTS 54 524 on parturition and lactation as evidenced by neonatal viability and growth after cross-fostering.

Dose: 0, 3.0 mg/kg orally by gavage as a single daily dose Day 17 of gestation through Day 21 of lactation. Vehicle deionized water. Concentration 0.3 mg/ml.

No. Animals: 25 animals per group (Groups 1 and 2). Mated Female Charles River Crl:CD VAF/Plus Rats.

F_1 offspring (although potentially exposed in utero and as neonates through nursing) were untreated.

Females were allowed to deliver. On lactation day 0 after delivery was completed and pups weighed, the pups were cross-fostered. Pups from an untreated dam were fostered to a treated dam and vice-versa. If no litter was available for cross-fostering the litter remained with its own dam (Groups 3 and 4).

Sacrifice - dams and pups - lactation day 21.

Group 1 = Control Dams/Control Pups - lactation day 0
Treated Dams/Control Pups - days 4-21 of lactation

Group 2 = Treated Dams/Treated Pups - lactation day 0
Control Dams/Treated Pups - days 4-21 of lactation

Group 3 = Control Non cross-fostered (7 animals) - no litter available
Group 4 = Treated Non cross-fostered (1 animal) - no litter available

F_0 Maternal Observation: 100% survival for control and treated. One treated dam sacrificed on day 25 due to non-delivery had a splenic cyst. Other antemortem findings were of a low incidence and not considered (by the sponsor) as treatment related. The only treatment-related bodyweight change (3%) in the 3.0 mg/kg was on day 7 which was prior to treatment.

In general there were no treatment-related differences noted between control and treated groups for maternal survival, antemortem observations, gestation and lactation body weights.

There was a greater number of non-gravid in the 3.0 mg/kg group (8/25) than in the control group (2/25).

F_0 Parturition and Litter Observation: Mean gestation length, similar for control and treated, was 22 to 23 days. The index for pup survival from cross-fostering to lactation day 4 was lower for treated pups cross-fostered to control dams compared to control pups cross-fostered to treated dams. Although not statistically significant the sponsor considers this finding to demonstrate that there was evidence of direct toxicity in pups born of treated dams since, after day 4, the number of deaths was comparable between control pups cross-fostered to treated dams and treated pups cross-fostered to control dams.

Compared to control pups, mean pup weights were lower (non sig.) for treated pups on day 0. Starting on lactation day 7 there was a small weight difference between treated pups cross-fostered to control dams and the control pups cross-fostered to treated dams; mean weights were 13.7 gms and 12.0 gms respectively. Similar findings were seen on day 21 with statistical significance for the mean pup weight of 40.5 gms for control males cross-fostered to treated dams compared to that of 46.9 gms for the treated males cross-fostered with control dams. Although not statistically significant mean pup weight was lower for the control female pups cross-fostered to treated dams compared to mean pup weights of treated females cross-fostered with control dams at the same interval.

Carcinogenicity Studies:

Palatability Study in the Charles River CD-1 Mouse:
Code MC 0030 dtd. Jan 1988
Batch: 3 as DW/E16) Q.A.: Present (Amend. 1,5 Aug 96).

Groups of 12M;12F immature, and mature, mice were allowed 14 days continuous access to BTS 54 524 diets containing concentrations of 85 to 115 ppm that were calculated to provide a level of 20 mg/kg; mature mice were maintained for an additional 14 days at the highest concentrations that mice given 20 mg/kg are likely to be given at any stage during a long-term feeding study, i.e. 160 ppm for males and 140 ppm for females. Controls were given untreated diet.

No deaths and no overt signs of reaction to treatment were noted. At the start of treatment mice showed transient lower weight gain, or small weight losses, and ate slightly less than controls.

Mature mice and immature males treated with BTS 54 524 reverted to normal weight gains quicker than immature females which showed normal weight gains beginning in the 2nd week. When the concentration was increased in week 3 for mature mice there was an initial suppression of weight gain which subsequently became similar to controls.

The initial slightly lower food intake persisted for up to 4 days. Immature treated females also showed slightly lower consumption during the 2nd week.

When the dietary concentration was increased for mature mice on day 14 there was an initial drop in food intake, however this change was still comparable with that of controls.

Changes in weight and food consumption were considered by the sponsor to be toxic effects rather than an indication of unpalatability. They concluded that a diet containing concentrations of BTS 54 524 up to 160 ppm in males and 140 ppm in females is palatable to mice.

Oral Carcinogenicity Study: Sibutramine Hydrochloride - Effects on Tumor Incidence in the Charles River CD-1 Mouse:

Report TX92006, T125, T125A. Code MC 0031. dtd. Jan 1992. Study began Jan 1987. Batch: No. 3, as DW/E16. Q.A.: Present.

Dose: 0, 0, 1.25, 5 or 20 mg/kg daily in the diet (2 control groups)

No. Animals: 52M;52F Charles River CD-1 mice per group.

Dose levels were reported to have been chosen on the basis of the 13-week and palatability studies.

Clinical signs, bodyweight and food consumption were recorded weekly. Bone marrow samples were collected at terminal autopsy and, where practicable, from mice dying or killed during study.

Sacrifice was after 95 weeks for males (when survival in one control group and in the high dose group approached 25%) and 104 weeks treatment for females. All mice were dissected and examined macroscopically. Microscopic examination was conducted on all tissues from the two control groups, the high dose group and on macroscopically abnormal tissues from mice given 1.25 or 5 mg/kg. Beginning week 27, the location, appearance and dimensions of all palpable tissue-masses were recorded weekly.

Achieved intakes, averaged over the study, were 1.25, 5.01 and 20.18 mg/kg daily for males and 1.25, 5.01 and 19.96 mg/kg daily for females.

Dietary formulation analysis showed overall mean concentrations to have been between 89.7 and 94.2% of intended.

Statistical analyses used combined data from the control groups, except for terminal myelograms where only one control group was examined. In addition, for tumor analysis, comparisons were also made (data not presented) against the individual control groups.

Results:

Survival was reported not to be adversely related to treatment since no statistically significant differences were revealed. The **percent survival for high dose males was lower than that for controls or treated,** i.e. control(2) - high dose being as follows: males 29, 39, 42, 33, 27%; females 44, 33, 40, 46, 50%. A number of high dose males had to be isolated from cage mates due to aggressive behavior or fighting injuries; they also had a slightly higher incidence of cutaneous lesions. Commonly observed signs with no difference in incidence from that of controls included; hair loss, body stains, swollen limbs, hunched posture and piloerection.

Palpable masses were fewer in high dose females and both male and female high dose animals had a lower mean number of palpable masses per animal.

High dose mice had lower bodyweights than controls. They were lower for the first 20 months in females and throughout study for males. Averaged over weeks 1 to 26, 27 to 52 and 53 to 78 bodyweights were significantly lower for high dose males and females at each period. Compared to combined controls the adjusted mean values were lower by 6 to 9% for males and 4 to 7% for females. Doses of 1.25 and 5 mg/kg did not affect bodyweights.

From week 46 onward high dose males generally ate slightly more than controls. This reached significance compared to controls during weeks 53 to 78.

Neither hematology nor bone marrow myelograms showed any apparent treatment related findings. A wide variation was seen in hematological parameters especially among mice killed or dying during the study. Compared to combined control data, platelet counts for high dose females were significantly higher. However, when compared against control groups individually, the difference was significant only from Control Group 1 with most of the individual values being within control range.

Myelograms from final autopsies showed high dose females to have significantly higher percentages of intermediate and late normoblasts and total erythroid cells, and significantly fewer myeloid cells. Since there was no significant change in the myeloid:erythroid ratio, the biological importance of such changes is uncertain. Although individual values were normal there were significantly fewer megakaryocytes in high dose females.

Findings were in general typical of those seen in long term animal studies and showed no apparent difference in incidences between control and treated.

Histologically there was a slightly increased incidence, but not severity, of serous cell vacuolation in male high dose salivary glands. Most of the wide variety of non-neoplastic lesions were attributable to ageing.

Major causes of morbidity and mortality were not treatment-related and included the following:

Thrombosis of the left and sometimes right atrium (mostly in males) - in several cases associated with myocardial degeneration and fibrosis, and with pulmonary alveolar histiocytosis.

Severe cases of chronic glomerulonephropathy (in both sexes).

Chronic skin lesions.

Urinary stasis (especially males) - depicted by distended and often hemorrhagic bladder, inflamed and edematous accessory glands, kidney pelvic and tubular distension due to back pressure. An urethral plug of eosinophilic material was noted in some cases. Amyloidosis (mostly in females) - in the kidney mostly as part of a generalized syndrome - other typical sites included ovary, intestine, lymph nodes and blood vessel walls of many tissues.

Neoplastic lesions: [See Tables in Statistical Review and Evaluation - attached.]

The overall incidence of benign and malignant tumors was not affected and there were no statistically significant increases in any individual type of tumors. The numbers of skin and mammary tumors were not affected.

Mid and high dose males had a lower incidence of adenocarcinomas of the secretory stomach which resulted in a statistically significant negative trend (p=0.988). [Adenocarcinoma was reported to be distinguished from hyperplasia by evidence of breaching of the muscularis mucosae (as opposed to invagination) with growth of glandular structures into the submucosa.] No statistical increase was reported for stomach secretory glandular hyperplasia.

Small islet-cell adenoma (classified as benign incidental tumor) was seen in the pancreas of 1 male control and 2 female high dose only. **Hemangioma (benign)** was seen in the uterus of **two high dose females** only.

A number of tumor types were **common in all groups**. Hepatocellular tumors, more common in males, were a notable cause of morbidity and mortality in males. Most of these tumors were single, however, multiple tumors were also seen. Hepatocellular carcinomas [controls(2)-high dose: males 8, 14, 9, 7, 10] in general were well differentiated with no evidence of metastasis - they were diagnosed by the presence of thick trabeculae and sheets of cells.

Both single and multiple foci were seen in lung adenomas and carcinomas. Focal differentiation into a sarcomatous pattern was seen in two carcinomas, but most had the typical papillary cellular arrangement. Carcinomas were generally well differentiated - diagnosis was by evidence of invasion into bronchi or spread via the bronchial tree. Some of the larger tumors were considered to have been fatal.

Lymphosarcomas were present mainly in females. A few were only in lymph nodes, thymus or cecum, however, the majority were widely metastasized and a few were leukemic.

Histiocytic sarcomas were common to females with some being confined to individual organs such as uterine cervix or to a few abdominal organs, however many had metastasized widely. A few myeloid leukemias were found three of which were myelomonocytic.

The majority of hemo-lymphoreticular tumors (especially those widely metastasized) found in preterminal mice were considered to have been fatal.

Skin sarcomas, most of which were poorly differentiated, were most common in males (in general comparable to controls). Some of these could be characterized as fibrosarcomas or myosarcomas. A few were multifocal and many were locally invasive. There was a tendency towards ulceration causing morbidity and mortality.

Palatability Study of BTS 54 524 in the Charles River CD Rat: Study Code RC 0076 dtd. May 1988. Batch: 3, as DW/E16 Q.A.: Present (Amend 1,5 Aug 96).

The object of this study was to determine the palatability of diet containing concentrations of BTS 54 524 calculated to achieve an intake of 20 mg/kg daily throughout a long-term feeding study in rats.

Groups of 12 per sex immature and mature Charles River CD rats were permitted 14 days continuous access to diets containing concentrations of 220 to 300 ppm BTS 54 524 which had been calculated to provide a level of 20 mg/kg daily. Then dietary concentrations were progressively adjusted until week 7 at which time they were equivalent to the highest concentrations that rats receiving 20 mg/kg daily would be likely to receive at any stage during a long term feeding study. The levels available throughout week 7 were 600 ppm for males and 450 ppm for females. Similar control groups received untreated diet.

BTS 54 524 caused an increased incidence of behavioral changes of the type due to CNS stimulation. Such changes included increased activity, excitability, vocalization and aggression during the first week. Except for immature treated females these changes became less prevalent with continued treatment. The incidence of skin lesions and hair loss was higher in treated rats, especially mature females, than in controls.

Beginning with the start of treatment, rats on 220 to 300 ppm lost weight and consumed less food than controls. After a couple of days for immature rats and up to a week or two for mature rats, food consumption and weight gain improved in all groups although food intake of immature males and weight gain of all treated groups stayed slightly lower than that of controls. The pattern of weight gain and food consumption was not changed when BTS 54 524 was increased to 600 ppm for males and 450 ppm for females (dietary concentrations necessary to achieve 20 mg/kg would increase over a long-term study in accordance with body weight changes). Thus the sponsor considered reductions in food intake to be toxic in nature rather than an indication of unpalatability and concluded that concentrations up to 600 ppm for males and 450 ppm for females are palatable to rats.

2-Year Oral Carcinogenicity Study: Sibutramine Hydrochloride - Effects on Tumor Incidence in the Sprague-Dawley CD Rat:
Study Code RC 0078 dtd. March 1992. Batch: 3, DW/E16. Study began April 1987; Report dtd March 1992. Q.A.: Present.

Dose levels for this study were reported to have been chosen on the basis of 14-day, 4-week and six-month repeated-dose oral toxicity studies and a palatability study. 9 mg/kg in this study was reported to be the maximum tolerated dose by virtue of reduced bodyweight in both sexes.

Dose: 0, 0, 1, 3, 9 mg/kg daily in the diet for 104 weeks.
(2 control groups)

No. Animals: 52M;52F per group

Clinical signs, bodyweight and food consumption were recorded weekly. [Bodyweight and food consumption were analyzed over 26-week periods up to week 78; beyond this point, intercurrent mortality prevented meaningful evaluation.] Begining week 27, the location, appearance and dimension of all palpable tissue masses was recorded weekly. Blood samples for hematological examination and bone marrow for myelograms were collected at terminal autopsy and, where practicable, from rats killed during the study.

Survivors were killed after 104 weeks treatment, subjected to a full macroscopic examination and tissue samples, including tissue masses, were taken and fixed. Histopathology was conducted on all tissues from rats in the two control groups and the high dose group, and on testes and macroscopically abnormal tissues from the 1 and 3 mg/kg groups.

Achieved drug intakes averaged over the study for males were 1.00, 3.00, 9.01 mg/kg daily and for females 1.01, 3.01 and 9.03 mg/kg daily.
Diets contained an average of 92-96% of intended concentrations.

Results: [See Tables in Statistical Review and Evaluation -attached.]

Survival showed no treatment-related effects - 53% of the males [controls(2) through high dose - 48, 44, 57, 65, 52%] and 54% of the females [controls(2) through high dose - 50, 48, 50, 64, 58%] survived the 104-week dosing period. [Renal failure was a more frequent cause of death in high dose males than in other groups.] Low and mid-dose male rats and mid- and high dose female rats showed a slightly better survival than controls at 104 weeks. Statistical analysis of survival throughout the study, however, showed no significant differences between treated and control groups.

Mid- and high dose females exhibited an increased incidence of behavioral changes consistent with CNS stimulation. These included increased activity and at the high dose only, increased vocalization and tenseness on handling. Four high dose females and 4 males(also 2+1 controls) showed increased aggression and at the high dose both sexes showed a slightly higher incidence of piloerection. High dose females also showed an increased incidence of cutaneous lesions, and red or swollen limbs and digits possibly in relation to CNS stimulation and aggression. High dose females also had a slightly higher incidence of protruding eyes (with no consistent associated histopathological finding).

Compared to controls fewer treated males had palpable masses. The number of masses per rat for high dose males was slightly lower and for high dose females very slightly higher.

Other signs (incidences similar to that of controls) were those commonly observed in lab rats and included hair loss, body stains, pallor and loss of co-ordination.

Although weights varied from week to week, significantly lower values were seen for all doses when bodyweights, averaged over weeks 1 to 26, 27 to 52 and 53 to 78 were subjected to statistical analysis. Bodyweights for both males and females showed a dose-related reduction. When the three periods were combined, it is reported that average body weights low through high dose were lower than controls by 3, 9, and 12% for males and 4, 10 and 13% for females.

Treated rats ate less than controls. Reductions in food consumption were generally related to bodyweight reductions. Food consumption averaged over weeks 1 to 26, 27 to 52 and 53 to 78 showed significantly lower values for all treated, except for low dose females during weeks 53 to 78.

Hematology values showed considerable variation reflecting age and disease status especially in those killed or dying during the study. Hematology and bone marrow myelograms were reported to show no treatment related changes. However, final autopsy showed significantly higher erythrocyte counts for mid- and high dose males, higher MCHC values for all groups of treated males and lower MCV and MCH values for mid- and high dose females. Differences were reported to be small. Myelograms at final autopsy showed significantly higher percentages of neutrophils for high dose males and females; there were no increases in neutrophil precursors or in the overall percentage of myeloid cells.

Autopsy showed treatment males to have a slightly lower incidence of subcutaneous masses, and mid- or high dose females to have a higher incidence of subcutaneous cysts, affirming palpation findings. High dose females had a higher incidence of swollen or thickened limbs. A higher incidence of distended urinary bladder and of enlarged prostate (histology showed prostatic inflammation) were seen in mid- and high dose males. Renal failure, the most frequent cause of death in high dose males was considered to be associated with urinary stasis and bladder obstruction. Other changes not associated with overt signs or histopathology findings included: a lower incidence of thickened or swollen livers in low and high dose males and mid or high dose females; a lower incidence of mottled livers in mid- and high dose females; a lower incidence of kidney cysts in mid- or high dose males; and in treated females a lower incidence of enlarged spleens.

Although not statistically significant the incidence of granulation tissue/granuloma and/or reactive bone and/or abscess was slightly higher in mid- and high dose surviving females.

The overall incidence of benign and malignant tumors was reported not to be affected by sibutramine.

However, there was an **increase in the incidence of benign interstitial-cell (Leydig cell) tumors (1, 5, 6, 6, control-high dose) of the testes (significant positive trend: p=0.013)** and a decrease in the incidence of mammary fibroadenomas (significant negative trend p=0.02) in treated males. The incidence of interstitial-cell hyperplasia was higher in mid-dose males. There was also a significant negative trend (p=0.04) for mammary fibroadenomas in female rats although the incidence in treated groups was within the range of the two control groups.

The following types of tumors appeared with a similar incidence among all groups:

Pituitary adenoma was the most common endocrine tumor with an incidence in males of 51% and 77% in females. A number of these tumors became large enough to cause compression of the brain, resulting in loss of condition and death.

Adrenal medullary tumors, benign and malignant, were seen in a few animals (mostly males). A few cortical tumors were also noted.

Pancreatic islet-cell adenomas and adenocarcinomas were seen predominantly in male rats.

Thyroid C-cell tumors, benign and malignant were present in small numbers.

Histiocytic sarcoma and lymphosarcoma (predominantly in males) were fatal in some cases. One case of monocytic leukemia was present.

Hepatocellular adenomas and carcinomas (a few were fatal) were present in small numbers.

Skin tumors showed a wide variety.
Some of the sarcoma/fibrosarcoma/fibromas became ulcerated, necessitating sacrifice. Keratoacanthomas were also present. These skin tumors were predominantly in males.

A variety of incidental non-neoplastic lesions were present most of which were attributable to aging. There was a higher incidence of prostate inflammation in high dose males.

Non-neoplastic lesions which were major causes of morbidity and mortality included the following: Renal failure not attributable to a single lesion in high dose males - associated with urinary stasis and bladder obstruction; chronic glomerulonephropathy in both sexes (majority of fatal cases were control males).

Sibutramine: Oncogenicity Study in Rats. Effects on the Testes.
dtd. March 1992.

With regard to the above CA study at 0, 1,3, 9 mg/kg in rats, there was an increase in the incidence of Leydig cell tumors in all treated groups, and a higher incidence of interstitial cell hyperplasia in the 3 mg/kg (but not 9 mg/kg) group.

Possible explanation offered by the sponsor: Interstitial cell adenomas are relatively common and of extremely variable incidence in rats but are rare in man. The incidence in treated rats in this study was within the background range for the strain, but was higher than the concurrent controls and background incidence at Boots. Interstitial cell adenomas in rats are associated with a variety of compounds none of which has been associated with testicular tumors in man. Neither Sibutramine nor the other compounds appear to have chemical or pharmacological features in common; tumors usually appear in old rats and are treatment rather than dose-related. The sponsor further indicates that limited work reported on the pathogenesis of the tumors suggests that important features are reduced plasma testosterone concentration and consequent increased pituitary-LH secretion, possibly together with suppression of the natural increase in plasma prolactin concentration.

Sibutramine Hydrochloride: Immunohistochemical Investigation of Pituitary Tissue from Male Rats.
dtd. May 1994 Study Code RC 0130. Q.A.: Present.

The 2-year carcinogenicity study in rats at doses of 1, 3, 9 mg/kg sibutramine in the diet resulted in a treatment, but not dose-related (see above) increase in the incidence of testicular interstitial-cell adenoma (testicular interstitial-cell hyperplasia also increased at 3 mg/kg).

Pituitary tissue selected from the male rats used in the completed oncogenicity study was examined by an immunohistochemical (immunoperoxidase) method, for differences in the numbers of pituicytes staining positively for gonadotropins and prolactin (PRL); The examination also included a subjective assessment of the overall stain intensity on each section.

Sections of fixed pituitary tissue from the following animals were examined for LH-, FSH- and PRL producing cells: 6 rats without testicular interstitial-cell hyperplasia or adenoma, but with reported histologically normal pituitary, from each control group and 1, 3, or 9 mg/kg sibutramine groups; all rats reported as having either testicular interstitial-cell hyperplasia or adenoma.

Results:

Staining intensity of LH and FSH appeared greater in treated than in control groups. Sibutramine treated rats had statistically significantly more pituitary cells staining for LH than controls; highest numbers were for 3 mg/kg rats but without interstitial-cell hyperplasia or adenoma. Treated rats also appeared to have more cells staining for FSH than controls. Only 3 mg/kg rats showed statistical significance.

The stain intensity for prolactin was in general similar in treated and control rats with no apparent statistically significant differences in numbers of positively-stained lactotrophs.

Staining characteristics of pituiticytes of rats with normal testes and those with hyperplasia or adenomas showed no obvious differences. Increased storage, but not necessarily increased secretion, can be identified by immunohistochemical techniques. It was assumed that increased storage reflected higher hormone circulating levels since pituiticytes were histologically normal.

Thus, the sponsor concluded that increased LH and FSH levels may at least be part of the mechanism of interstitial-cell hyperplasia and adenoma associated with long term sibutramine treatment of male rats.

Plasma LH was not measured.

Mutagenicity Studies:

BTS 54 524 (Sibutramine)

Q.A.: Signed by QA Manager as in compliance with GLPs (no inspection dates).

In-Vitro Bacterial Mutagenicity Test - Ames Test: Report TX81074 dtd. Dec 1981 Batch: TL2

Ames test conducted using Salmonella typhimurium TA 1535, TA1537, TA1538, TA98 and TA100 in the presence and absence of an in vitro liver microsomal metabolic activation system (S-9) derived from Aroclor 1254-induced Charles River Wistar rats. Dimethyl sulfoxide was the solvent. Doses assayed were up to maximal dose levels allowing adequate growth of the indicator strains.

BTS 54 524 did not possess mutagenic activity in the tester strains up to 500 μg/plate in the presence and 15.6 μg/plate in the absence of S-9.

In-Vitro Bacterial Mutagenicity Testing: Report TX84051 dtd. July 1984 Batch 1/R4,

Ames test: BTS 54 524 was negative in this test at a maximum dose-level permitted by the antibacterial activity of the drug, i.e. 625 μg/plate in the presence of S-9 and 39 μg/ml in the absence of S-9.

In-Vitro Mammalian Cell Forward Mutation Assay: Report TX85114 dtd. Dec 1985. Batch 2 P21/37)

Replicate assays were conducted with Chinese hamster V79 cells in both the presence and absence of an exogenous metabolic activation system comprising a post-mitochondrial, S-9 fraction of livers from Charles-River Wistar rats pretreated with Aroclor 1254, supplemented with cofactors for NADPH generation.

When tested at the maximum concentrations permitted by cytotoxicity, BTS 54 524 was not mutagenic at 20 μg/ml in the absence of S-9, or up to 80 μg/ml in the presence of S-9. [There was an isolated non-repeatable increase at 40 μg/ml.]

In-Vitro Human Lymphocyte Clastogenicity Testing: Report TX85115 dtd. Dec 1985. Batch: 2 P21/37)

BTS 54 524 was examined for induction of human lymphocyte chromosome aberration in vitro in the presence and absence of metabolic activation system, S-9, derived from the livers of male rats pre-treated with Aroclor 1254. The concentration was limited to 250 μg/ml due to the solubility of BTS 54 524. In the absence of S-9 levels above 150 μg/ml were cytotoxic.

The incidence of chromosome aberrations in BTS 54 524-treated lymphocytes was similar to that seen in the negative control groups at dose levels up to the above maximal levels, indicating that BTS 54 524 was without in vitro clastogenic activity against cultured human lymphocytes.

Micronucleus Assay in MF1 (OLAC) Mice: Report TX86024 dtd Jun 1986. Batch: 2 P21/37).

Groups of 12M;12F mice were given BTS 54 524 at single oral doses of 62.5, 125 or 250 mg/kg (maximum tolerated dose). Purified water acted as a negative control and cyclophosphamide at 75 mg/kg as a positive control.

A second assay was carried out in which groups of 30M;30F mice were given single oral doses of 125 and 250 mg/kg BTS 54 524. Controls were as above.

Smears from the femur bone marrow were examined for the presence of micronuclei; the number of micronuclei per 1000 polychromatic erythrocytes per animal was determined.

In the first assay, all males (except for the one survivor of the high dose 48 hr. sampling) showed micronuclei numbers similar to that of the negative controls. At 62.5 or 125 mg/kg females showed no increase in the incidence of micronuclei. A slightly raised value ($P<0.05$) was seen with 250 mg/kg at 72 hrs.

The second assay showed males and females to have slight increases in the numbers of micronuclei ($P<0.05$) in the high dose after 24 hrs. Findings for control vs high dose at 24, 48, 72 hrs. were: male 0.24 ± 0.17, 0.19 ± 0.11, 0.20 ± 0.12 vs 0.30 ± 0.10, 0.7 (sig), 0.1; female 0.10 ± 0.11, 0.28 ± 0.12, 0.18 ± 0.10 vs 0.23 ± 0.12, --, 0.37 ± 0.12 (sig.) - Values for 62.5 and 125 mg/kg were not significant. No significant effects were reported on the ratio of polychromatic to mature erythrocytes at any dose level for either sex.

Both assays showed marginal increases in the incidence of micronuclei in some mice at 250 mg/kg, however it occurred at different sampling times (24 and 72 hrs.). There did not appear to be a dose relationship and the increased numbers of micronuclei occurred only in groups showing a marked reduction in hemopoiesis and/or a number of deaths (survival less than 50% in some cases).

The sponsor concluded that the maximum tolerated doses of the drug were without in vivo clastogenicity in the mouse micronucleus assay.

Sensitization Studies:
Skin Sensitization Study of BTS 54 524 in Hartley/Dunkin Guinea-Pigs:
Report TX85059. 8571D/BTS 204/SS May 1985. Batch: 1/R4, P24/59. Q.A.: Present.

Guinea-Pig maximization test: E. Magnusson and A.M. Kligman (1970) in "Allergic contact Dermatitis in the Guinea-Pig: Identification of contact allergens", published by C.C. Thomas, Springfield, Illinois.

Induction - Intradermal injection: 0.01% w/w in water for irrigation. Topical application: 50% w/w in distilled water. [50% w/w was not irritant. However, concentrations above 50% were too dry for satisfactory topical application.]

Challenge - 50% and 25% w/w in distilled water.

1/20 test guinea pigs showed evidence of delayed contact hypersensitivity, with the remaining 19/20 being negative. Based on the one positive animal, the drug was classified as a weak (Grade I) sensitizer.

Local Irritancy Studies:
Skin Irritancy in Albino Rabbits:
Report TX85032. 841057D/BTS 20/SE Feb 1985. Batch: 1/R4 Q.A.: Present.
OECD Guideline for Testing of Chemicals No. 404:
"Acute Dermal Irritation/Corrosion"

A single 4-hr. semi-occluded application of BTS 54 524 to intact rabbit skin did not produce any observable dermal irritation.

Irritant Effects on the New Zealand White Rabbit Eye:
Report 8521D/BTS 203/SE dtd. Apr 1985. Batch: 1/R4 Q.A.: Present. OECD Guideline No. 405.

A 59 mg aliquot of BTS 54 524, the weight that occupied a vol. of 0.1 ml, was placed into the lower everted lid of one eye of each animal; the contralateral eye served as a control.

A positive response was seen in all three rabbits. The death of one animal 10 days after instillation was not considered by the sponsor to have been treatment related. Corneal opacities (still present after 21 days in 2 survivors), iridial inflammation, and well-defined conjunctival irritation, accompanied by areas of eyelid necrosis, were seen in all 3 rabbits.

Special Studies: [Including Metabolites, Enantiomers, Impurities.]

unless otherwise stated.
BTS 54 524 - sibutramine
BTS 62 930 - the (+) enantiomer of sibutramine
BTS 62 931 - the (-) enantiomer of sibutramine
BTS 54 354 - is an impurity and a metabolite of BTS 54 524 in mice and rats
BTS 54 505 - 1-[1'-(4-chlorophenyl) cyclobutyl]-3-methylbutylamine hydrochloride found as an impurity in production batches of BTS 54 524 (sibutramine) is the hydrochloride salt of BTS 58 726, a compound that has shown indications of possible weak direct mutagenic activity in the Ames test [but subsequently found not to be genotoxic in mammalian-cell based systems].

Amendment: At the time original study was conducted. BTS 54 505 had been identified as an impurity in some batches of BTS 54 524. A memorandum dtd. 29 June 1987 from Dr. Brown to Mrs. Austin [Boots Pharmaceuticals, England] indicates that BTS 54 505 should not now be regarded as an impurity. [Mutagenicity studies, however, were conducted with BTS 54 505 in 1993.]

BTS 58 726 - 1-[1-(4-chlorophenyl)cyclobutyl]-3-methybutylamine. Crude BTS 58 726 (86-87% pure) is the stage 3 intermediate in the BTS 54 524 (sibutramine) manufacturing process. BTS 58 726, in its pure form, is a metabolite of BTS 54 524 in rats.
BTS 59 482 - A metabolite of BTS 54 524 in rats.
BTS 64 472 - c-3-(1-amino-3-methylbutyl)-3-(4-chlorophenyl)-r-1-cyclobutanol fumarate, the aglycone of Metabolite 5 of BTS 54 524.
BTS 65 400 - 4-amino-4-[1-(4-chlorophenyl)cyclobutyl]-2-methylbutan-1-ol hydrochloride, the aglycone of Metabolite 6 of Product BTS 54 524.

Metabolites 5 and 6 are the major human metabolites (found in a ratio of 2:1, respectively in human plasma).

Acute Oral Toxicity Studies:

BTS 62 930

Rats: Charles River CD - Report TX91022 (RA 0202) dtd Mar 1991. Lot: TL2 Q.A.: Reported as in accordance with principles of GLP's (UK).

Groups of 3M;3F were given single oral doses of 1.5, 3.125, 12.5 or 50 mg/kg BTS 62 930 in Purified Water BP, at a dose vol. of 1.0 ml/100 g bodyweight.

Excitability was seen at the lower doses and the two higher doses showed marked CNS stimulation within 1 hr after dosing including increased activity, rapid gait, dilated pupils, excitability and exaggerated reactions to noise or movement; some high dose rats also showed general non-specific signs of toxicity. High dose rats showed stereotyped sniffing behavior; one male and one female had to be killed the day after dosing due to lesions resulting from excessive grooming.

Body weights of the two higher doses were affected and overall weight gain was retarded at all dosages.

Overt signs were reported to be qualitatively similar to those with sibutramine, but quantitatively more marked. [Actual aggressive behavior and enlarged livers were reported for sibutramine only.]

BTS 62 930 vs Sibutramine: No-effect level not determined. Lowest dosage eliciting overt signs 1.5 vs 12.5 mg/kg. Highest non-lethal dosage 12.5 vs 50 mg/kg. Lowest lethal dosage 50 vs 100 mg/kg. Highest dosage with survivors 50 vs 150 mg/kg.

Monkeys: Cynomolgus (Macaca fascicularis):
Project 650309 Report TX91042 dtd. May 1991. Batch: TL2. Q.A.: Reported as in accordance with principles of GLP's (UK).

Single oral doses of 10, 3 and 15 mg/kg BTS 62 930 were given to a pair of monkeys in Purified Water BP, at 4 ml/kg with a 14 day observation period between doses. Another pair of monkeys received vehicle as controls. Electrocardiograms were recorded pretrial and 24 h after each administration. Hematology and clinical chemistry were carried out, as well as histology on liver with gall bladder, kidneys, testes with epididymides, brain and any macroscopically abnormal tissue.

A single 3 mg/kg dose produced no overt signs. At 10 and 15 mg/kg signs of CNS stimulation were consistent with an exaggerated pharmacological effect. Signs including hyperactivity, pupillary dilatation and agitation persisted up to 30 hrs. with 10 mg/kg and up to 84 hrs. with 15 mg/kg.

Hematology showed no apparent treatment-related effects, there was however, a mild increase in reticulocytes for both animals after the 4 and 10 mg/kg doses.

Effects on the liver were indicated by changes in blood chemistry (AST, ALT, total bilirubin and triglycerides increases) and both monkeys showed mild hepatocellular fat vacuolation histologically and slightly reduced liver weights.

Electrocardiograms (unanesthetized) pretrial and 24 h after each dose showed no treatment-related changes.

Both monkeys showed a mild increase in reticulocytes after dosing at 3 and 10 mg/kg.

Changes were reported to be qualitatively similar to those seen with sibutramine, but quantitatively more marked.

<u>BTS 62 931</u>

Rats: Charles River CD - Report TX91021 dtd. March 1991. Lot: TL2 Q.A.: Reported as in accordance with principles of GLP's (UK).

3M;3F rats per group were given single oral doses of 12.5, 50, 200, 400 mg/kg BTS 62 931 in Purified Water BP at 1.0 ml/100g body weight.

12.5 mg/kg produced no overt signs. At 50 mg/kg and above overt signs of CNS stimulation were seen. General non-specific signs of toxicity were also seen. 200 and 400 mg/kg rats displayed stereotyped sniffing behavior. Some groomed excessively with resulting lesions with one 200 mg/kg male and all males and one female at 400 mg/kg were killed two days after dosing. 50 mg/kg rats recovered overnight, 200 mg/kg males recovered within two days and 200 or 400 mg/kg returned to normal within 5-6 days.

Low weight gain or overnight weight loss was seen at 50 mg/kg and above. In general weight gains were lower in some males of each group and in some females in the two higher dose groups.

Macroscopic findings at autopsy (other than skin and related lesions) were not considered treatment-related.

It is reported that overt signs were qualitatively similar to that of sibutramine, but quantitatively less marked with the time of onset tending to be later with BTS 62 931.

Monkeys: Cynomolgus - [redacted].
Report TX91041 dtd. May 1991. Batch: TL2. Q.A.: Reported as in accordance with Standard Operating Procedures (UK).

Single oral doses of 10, 20, 30, 50 and 75 mg/kg BTS 62 931 in Purified Water BP (4 ml/kg) were given to a pair of monkeys. An observation of 14 days was allowed between successive doses.

Doses of 30 mg/kg and below produced no overt toxic signs. 50 and 75 mg/kg produced signs of CNS stimulation consistent with exaggerated pharmacological effects. Behavior was normal 24 hours after 50 mg/kg and 48 hours after 75 mg/kg.

Body weights showed no adverse effects.

There were little or no effects on food consumption except following dosing at 75 mg/kg the female showed zero food consumption.

Hematology showed no apparent treatment-related effects.

After 75 mg/kg the female had increased concentrations of serum bilirubin and triglyceride which returned to normal by day 8. The female liver had pale foci (mainly of parasitic origin) and histology of this animal showed mild hepatocellular fat vacuolation.

Organ weights were in general within range of normal.

Electrocardiograms were recorded for each animal (unanesthetized) during the pretrial period and at 24 h after each dose administration.

In general no effect was detected at doses up to 30 mg/kg and changes produced by BTS 62 931 were reported to have been qualitatively similar to those seen with sibutramine, but quantitatively less marked.

BTS 54 354

Mice: Charles River CD-1 - Report TX86008 dtd Feb 1986. Batch/Lot: TL4 Q.A.: Signed QA Manager as incompliance with FDA GLPs (no inspection dates).

Dose: Single oral at 12.5, 45, 100, 150 mg/kg in 0.4% Cellosize solution with a 14 day observation period to groups of 3 males.

Within ½ to 1 hour overt toxicity signs included increased activity, excitability, increased sensitivity to handling, noise and touch, fighting and aggressive behavior, piloerection, body stains, rapid gait and rapid respiration. At 45 mg/kg or more signs included ataxia and at 100 or 150 mg/kg a low hunched body, walking on tiptoe, noisy respiration and stereotyped behavior which included excessive grooming leading to sores. At 150 mg/kg signs also included irregular respiration, increased salivation, body stains, leaping gait, Straub tail and convulsions.

Two moribund animals were killed one on 150 mg/kg and one on 100 mg/kg. Survivors at 12.5 and 45 mg/kg recovered in 5 days and those at 100 and 150 mg/kg at 7 days.

Autopsy of the two dead animals showed GI irritation with a pale mottled liver in the one on 100 mg/kg. Those killed at the end of study showed no treatment-related findings.

The sponsor concluded that the toxicity in mice of BTS 54 354 was probably similar to that of BTS 54 524 (sibutramine).

BTS 54 505

Mice: Charles River CD-1 - Report TX86007 dtd. February 1986. Q.A.: Signed QA Manager as incompliance with FDA GLPs (no inspection dates). Single oral dose levels of 9, 50, 150, 200 mg/kg based on those used in a previous study with BTS 54 524 were given to Charles River CD-1 mice in 0.4% Cellosize solution. The observation period was 14 days.

Within ½ to one hour all groups showed overt signs of toxicity including excitability, rapid gait, increased sensitivity to handling, aggressive behavior, and an increased incidence of piloerection and body stains. 50 mg/kg and more produced increased activity, ataxia and stereotyped behavior, including excessive grooming that led to bald areas and sores. One 200 mg/kg mouse was killed on the day of dosing due to excessive grooming resulting in a large sore on the neck. At 150 and 200 mg/kg signs included increased salivation, convulsive movements, some with Straub tail, leaping gait, rapid or uneven noisy respiration, pale eyes, low hunched body position and walking on tiptoe.

General recovery was within overnight for the lower two doses and within 4-5 days for the two higher dose levels. No macroscopic abnormalities were seen at autopsy.

It is reported that overt signs of toxicity were qualitatively similar to that previously seen with BTS 54 524. However, the incidence at the same dose levels showed variation - marked behavioral changes were seen with 9 mg/kg BTS 54 505 but not with 12.5 mg/kg BTS 54 524.

BTS 58 726

Rats: Report TX 87092 dtd Aug 1987. (Reported as not subject to Q.A.)

Groups of 3M;3F Charles River CD rats received single oral doses of 3, 6, 12.5, 25, 50 or 100 mg/kg, and 2 control groups received 0.4% aqueous Cellosize solution vehicle with a 14-day observation period.

Signs of toxicity were seen within ½ hour. At 3 mg/kg the only sign was increased salivation in one rat. At 6 mg/kg or more there was evidence of CNS stimulation and excessively stained coats. One 100 mg/kg female had stereotyped behavior the day after treatment and had to be killed due to an extensive lesion due to excessive grooming. On high dose male also had a bald area and small lesion suggestive of stereotyped behavior. Overnight recovery was generally seen with 3, 6 and 12.5 mg/kg; by the second day 25 and 50 mg/kg rats recovered and survivors on 100 mg/kg returned to normal by day 3.

One 25 mg/kg male and all at 25 mg/kg or more lost body weight. For females, one on 6 mg/kg and most given 12.5 mg/kg or more lost more weight than controls. Weight losses were regained quickly and overall weight gain for survivors was comparable to controls.

The high dose animal that died showed macroscopic evidence of GI irritation, a pale spleen and a skin lesion. At the Day 14 autopsy one 12.5 mg/kg male had a red thymus due to an area of capillary hemorrhage.

Reported that the toxicity of single oral doses of BTS 58 726 in rats is qualitatively and quantitatively similar to that of BTS 54 524.

BTS 64 472

Mice: Charles River CD-1 Mice. TX88029 (MA 0074) dtd March 1988. Batch TL2 [Test Lot contained 96.16% of BTS 64 472 and 3.76% of its geometric isomer BTS 64 473.] Q.A.: Signed by QA Manager as monitored for GLP compliance (no inspection dates).

Groups of 3M;3F mice were given single oral doses of BTS 64 472 at 25, 50 or 100 mg/kg in solution in Purified Water BP, or at 400, 800 or 1330 mg/kg in suspension in 0.4% Cellosize solution. There was a control group for each vehicle with an observation period of 14 days.

One 50 mg/kg female and most mice at 100 mg/kg and up had dilated pupils. At 100 mg/kg and up there were signs of CNS stimulation, including increased activity, excitability, spontaneous vocalization, rapid respiration, restless behavior and rapid gait. There was also increased urination, stereotyped behavior. 400 mg/kg and above were ataxic and showed non-specific signs. Males on the two higher doses were aggressive. 1330 mg/kg females had increased salivation and stereotyped behavior - 1M;1F had to be killed on the day of dosing due to stereotyped behavior induced lesions. One 1330 mg/kg M on day 1 was killed moribund - pale areas on liver and red streaks on gastric mucosa. Recovery of the 1330 mg/kg mice persisted until day 8 for some; most had generally recovered by day 1 (50 mg/kg), day 2 (100, 400 and 800 mg/kg) or day 5 (1330 mg/kg). Day 14 autopsy showed no treatment-related findings.

Findings were mainly exaggerated pharmacological effects. No-effect dosage - 25 mg/kg; Lowest dosage eliciting overt signs - 50 mg/kg; Highest non-lethal dosage - 800 mg/kg; Lowest lethal dosage - 1330 mg/kg; Highest dosage with survivors - 1330 mg/kg.

Rats: Charles River CD Rats. TX88028 (RA 0182) dtd Mar 1988. Batch TL2. Q.A.: Present (UK).

Groups of 3M;3F rats were given single oral doses of BTS 64 472 at 25, 50, 100 or 200 mg/kg in soln. Purified Water BP, or 400 or 800 mg/kg in suspension in 0.4% Cellosize solution. Two control groups received one or the other vehicle with observation for 14 days.

Most had dilated pupils at 25 mg/kg or greater. Females of all groups and 50 mg/kg or greater males showed excitability and exaggerated reactions to handling. Additional signs of CNS stimulation seen after 50 mg/kg or more

included increased activity, exaggerated reactions to noise, restless behavior, rapid respiration and rapid gait and non-specific signs of toxicity. Stereotyped behavior was seen at 200 mg/kg or more. One 800 mg/kg female had tremor, Straub tail and clonic convulsions, and twitching tail. At 800 mg/kg stereotyped behavior led to lesions - resulting in 2M;2F being killed - evidence of GI irritation.

Except for some 400 and 900 mg/kg rats with lesions and sparse hair until the end of study most generally recovered by day 1 (25, 50 mg/kg) day 2 (100 mg/kg), day 3 (200, 400 mg/kg) or day 4 (800 mg/kg).

Transient bodyweight losses or retarded weight gains were slight at 50, 100, 200 mg/kg, and marked and more prolonged at 400 and 800 mg/kg. Weight gain the second week was normal.

Findings were mainly exaggerated pharmacological effects. No-effect dosage - not determined; Lowest dosage eliciting overt signs - 25 mg/kg; Highest non-lethal dosage - 400 mg/kg; Lowest lethal dosage - 800 mg/kg; Highest dosage with survivors - 800 mg/kg.

BTS 65 400

Mice: Charles River CD-1 - TX88084 (MA 0076) dtd August 1988. Batch TL1 Q.A.: Present (UK). Groups of 3M;3F mice were given BTS 65 400 in single oral doses of 6, 10.5, 50, 100 mg/kg in aqueous 0.4% Cellosize solution. Controls received vehicle. Observation was for 14 days.

6 mg/kg mice showed no signs of treatment. At 10.5 mg/kg or more, toxicity was evident within one hour and included excitability, aggressive behavior and rapid gait. Further signs of CNS stimulation after 50 or 100 mg/kg included increased activity, spontaneous vocalization, exaggerated reactions to noise or movement and restless behavior, as well as stereotyped behavior which led to skin lesions - within 2 hours two 100 mg/kg mice had to be killed. Recovery - 10.5 and 100 mg/kg survivors generally within 2 days; at 50 mg/kg within 4 days.

6, 10.5 and 50 mg/kg body weight gains were generally similar to controls. Two 100 mg/kg survivors lost weight slightly overnight after dosing, but were similar to controls during the remainder of the study.

Except for skin lesions there were no treatment-related macroscopic autopsy findings.

Findings were mainly exaggerated pharmacological effects. No-effect dosage - 6 mg/kg; Lowest dosage eliciting overt signs - 10.5 mg/kg; Highest non-lethal dosage - 50 mg/kg; Lowest lethal dosage - 100 mg/kg; Highest dosage with survivors - 100 mg/kg.

Rats: Charles River CD - TX88085 (RA 0188) dtd August 1988. Batch TL1 Q.A.: Present (UK).

Groups of 3M;3F rats received single oral doses of 12.5, 25, 100, 400 mg/kg BTS 65 400 in aqueous 0.4% Cellosize solution. Two control groups received vehicle alone. Observation was 14 days.

No signs of treatment were seen at 12.5 mg/kg. Within one hour the majority of 25 mg/kg and above rats showed signs of CNS stimulation, including increased activity, excitability, exaggerated reactions to noise or movement, rapid gait, dilated pupils, and excessive urination. At 100 mg/kg and above there were signs of rapid breathing, spontaneous vocalization and soft, pale feces. At 400 mg/kg some rats had Straub tail, increased lacrimation, dull eyes and other non-specific signs of toxicity and all showed stereotyped behavior with excessive grooming which led to skin lesions and to 2 females having to be killed the day after dosing. Recovery to normal was overnight for 25 mg/kg, and generally within 2 days for 100 mg/kg and 400 mg/kg survivors.

Bodyweight loss was seen for 100 mg/kg females and the 400 mg/kg female survivor. The 400 mg/kg female which was killed early had a marked weight loss. Two 400 mg/kg males had slightly reduced weight gain.

Except for skin lesions there were no apparent treatment-related macroscopic findings.

Findings were mainly exaggerated pharmacological effects. No-effect dosage - 12.5 mg/kg; Lowest dosage eliciting overt signs - 25 mg/kg; Highest non-lethal dosage - 100 mg/kg; Lowest lethal dosage - 400 mg/kg; Highest dosage with survivors - 400 mg/kg.

Additional Acute Toxicity Studies: Single oral dose studies of products (identity?) [BTS 54 580 (TX87002); BTS 55 126 (TX87003); BTS 61 934 (TX87004); BTS 60 430 (TX87055); BTS 56 705 (TX88018)] listed as possible impurities were carried out at doses up to 170 - 200 mg/kg. Signs appeared to be qualitatively similar to quantitatively less severe or of a similar order to that of sibutramine.

Special - Mutagenicity Tests of Metabolites etc.: (Metabolite List - See p.45)

Q.A.: Signed QA Manager as incompliance with GLPs (no inspection dates), unless otherwise indicated.

BTS 54 354

Ames Test: Report TX85099 dtd.Oct 1985. Batch TL4

BTS 54 354 induced reproducible increases in the numbers of TA1535 revertant colonies which, at a dose level of 156 μg/plate in one of the two assays conducted with this indicator strain, amounted to more than double the value of the negative control. Mutagenic activity was more pronounced in the absence of S-9. TA1537, TA 1538, TA98 and TA100 produced no evidence of mutagenicity.

According to the sponsor, these findings suggest that BTS 54 354 shows indications of **very weak direct base-pair substitution bacterial mutagenicity**.

In Vitro Mammalian Cell Mutation Assay: Report TX87020 dtd. Mar 1987. Batch TL 5.

Chinese hamster V79 cells - test for induction of 6-Thioguanine resistance a method which identifies mutagens which bind covalently to DNA.

BTS 54 354 showed no evidence of mutagenicity in Chinese hamster V79 cells when tested at concentrations up to 100 μg/ml, the maximum level allowed by cytotoxicity.

In Vitro Mammalian Cell DNA Repair Assay: Report TX87021 dtd. March 1987. Batch TL4 HeLa S3 cells - Test for induction of unscheduled DNA synthesis.

Concentration 10^{-7}M to 10^{-3}M - overt toxicity was seen with the highest concentration, thus in a subsequent assay the maximum concentration was restricted to 5 x 10^{-4}M BTS 54 354.

Isolated increases in DNA-repair activity were seen at 10^{-7}M in the absence of S-9, and at 10^{-5}M in its presence; that resulted in statistically significant differences from negative control. Another assay at 10^{-6}M in the absence of S-9 and 10^{-4}M in the presence of S-9 showed increases of the same order. A 3rd assay over a narrower range of concentrations showed no test agent-induced DNA-repair activity in the presence of S-9, but an isolated increase at 1.25 x 10^{-6}M in the absence of S-9. Magnitudes were similar to the first and second assays.

These assays showed **isolated instances of increased DNA repair activity**, however, findings were **inconsistent** and there was **no dose-response relationship**. The sponsor thus concluded that BTS 54 354 did not exhibit genotoxic potential in this system.

In Vitro Human Lymphocyte Clastogenicity Assay: Report TX87022. February 1987. Batch TL5. Test for in vitro induction of chromosome aberrations in cultured human lymphocytes.

No statistically significant increases in chromosome aberrations (including or excluding gaps) were seen when lymphocytes were exposed in the absence of S-9 metabolic activation to concentrations of BTS 34 354 up to 50 μg/ml (elicited marked cytotoxicity - greater than 50% decrease in mitotic index).

Micronucleus Assay in Mice (MN 0031): Report TX93101. dtd. August 1993. Batch TL7. Q.A.: Present (UK).

10M;10F female Charles River CD-1 mice per group were given single oral doses of BTS 54 354 at 25 or 50 mg/kg; 20M;20F were given 100 mg/kg (dose selected on the basis of an acute oral toxicity study). Negative controls received 0.4% aqueous Cellosize and positive controls the clastogen cyclophosphamide. Animals were killed at 24 or 48 hrs. and smears were prepared from femur bone marrow.

Within two hours BTS 54 354 animals showed signs of increased activity, excitable behavior, aggressive behavior (predominately males) tremor and tonic convulsions. Five males died at 100 mg/kg, 4M at 50 mg/kg and 1M at 25 mg/kg.

Significant increases were induced by the positive control, cyclophosphamide, but there were no significant increases in the incidence of micronuclei with 100 mg/kg BTS 54 354, thus BTS 54 354 showed no in vivo clastogenicity in the mouse micronucleus assay.

Autoradiographic Detection of DNA Repair Induced In Vivo in Rat Hepatocytes: Ltd. Report TX93177 BTS 278/931855 dtd. November 1993. Batch TL7. Q.A.: Present.

Single 50 and 150 mg/kg (determined in preliminary toxicity test) oral doses of BTS 54 354 were given to SPF outbred albino Hsd/Ola Sprague-Dawley rats to assess induction of DNA repair in hepatocytes.

Positive controls (dimethylnitrosamine, 4 mg/kg or 2-acetylaminofluorene, 50 mg/kg) showed a large and highly significant increase ($P<0.001$) in the net nuclear grain count which was accompanied by a large increase in the gross nuclear grain count. BTS 54 354 did not produce any substantial increases in either the gross nuclear grain count or the net nuclear grain count (i.e. the gross nuclear grain count minus the cytoplasmic grain count) at any dose level at either sampling time (2 or 14 hr. expression).

The conclusion was that BTS 54 354 did not elicit any evidence of DNA-damage in rat liver in this (in vivo) test system.

BTS 54 505 (See p. 45)

Ames Test: Report TX87015 dtd. February 1987. Batches TL3 and CTD 6999.

Results indicate that BTS 54 505 is a **weak direct base-pair substitution mutagen in the Ames test.**

In Vitro Mammalian Cell Mutation Assay: Report TX87016 dtd February 1987.

BTS 54 505 was reported to show no evidence of mutagenicity in Chinese hamster V79 cells [as indicated by forward mutation to 6-thioguanine (6-TG) resistance] at concentrations up to 50 µg/ml which was the maximum concentration permitted by its cytotoxicity. [A small increase in mutant frequency was seen at 50 µg/ml only - only marginally higher than control.]

In Vitro Mammalian Cell DNA Repair: Report TX87017 dtd. February 1987. Batch CTD 6999. BTS 54 505 was examined for induction of DNA-repair in HeLa S3 cells.

At the dose levels tested (max. 5×10^{-4}M), there was no significant increase in DNA-repair, thus the sponsor concluded that BTS 54 505, in the absence of S-9, does not have genotoxic potential in this test system.

In Vitro Human Lymphocyte Clastogenicity Assay: Report TX87018 dtd. February 1987. Batch CTD 6999. BTS 54 505 (up to 40 µg/ml) was examined for in vitro induction of chromosome aberrations (including or excluding gaps) in cultured human lymphocytes. The finding was negative.

Micronucleus Assay in Mice (MN 0030): Report TX93100 dtd. August 1993. Batch TL5. Q.A.: Present (UK).

Signs within two hours after dosing (25, 50, 100 mg/kg) included increased activity, excitable and aggressive behavior, tremor and convulsions. Deaths included 7M on 100 mg/kg, 1M on 50 mg/kg and 2M on 25 mg/kg.

The PCE:NCE ratio for the 100 mg/kg BTS 54 505 group at 24 hours was slightly higher than concurrent controls and the background control range, suggesting some evidence of treatment-induced bone-marrow toxicity. 100 mg/kg BTS 54 505 produced no significant increases in the incidence of micronuclei.

Autoradiographic Detection of DNA Repair Induced In Vivo in Rat Hepatocytes: Report TX93179 BTS 277/931845 dtd Nov 93. Batch TL5. Q.A.: Present.

The conclusion was that BTS 54 505 did not show any evidence of DNA-damage in the rat liver in this in vivo test since there were no substantial increases in either gross or net nuclear grain count at either dose level (50 and 150 mg/kg) at the 2 or 14 hour sampling times.

BTS 58 726 (See p. 45)

Ames Test: TX87010 dtd August 1987. Crude (86-87%) BTS 58 726 Batch 6743

In both the presence and absence of S-9 BTS 58 726 gave negative results with TA1535, TA1537, TA98 and TA100. Increased numbers of bacterial colonies were seen with TA1538 (but only at 39 and 78 µg/plate (the two lowest doses examined). On further testing with the same batch (CD6743) and batch CTD 6955 no evidence of mutagenic activity was seen in any of the tests.

The sponsor thus concluded that crude BTS 58 726 was without reproducible bacterial mutagenicity in the Ames test at dose levels up to the maximum allowing adequate growth of the bacterial indicator strains i.e. 312 µg/plate.

Ames Test: TX87011 dtd Feb 1987. Pure BTS 58 726 Batch TL1 and CTD 6962/LM/TOX/1. Range (up to 100 µg/plate for subsequent TA1535 assays).

In the absence of S-9, BTS 58 726 induced reproducible small increases in the numbers of TA1535 revertant colonies (maximum - approached twice the negative control). No evidence of mutagenic activity was seen in the presence of S-9 or against the other indicator strains.

Such findings are reported to suggest that **pure BTS 58 726 may have very weak direct base-pair substitution mutagenicity in the Ames test.**

Mammalian Cell, Chinese Hamster V79, Assay: TX87012 dtd Feb 1987. Pure BTS 58 726 Batch CTD 6962/LM/TOX/1

No evidence of mutagenicity was seen in V79 cells when tested up to 25 µg/ml (maximum level permitted by its cytotoxicity).

In Vitro Mammalian Cell (HeLa S3) Cell DNA Repair Assay: TX87013 dtd Feb 1987. Batch CTD 6962/LM/TOX/1

DNA-repair activity showed no statistically significant increase in either assay at the levels tested. Thus this test showed no genotoxic potential in the absence of S-9.

In Vitro Cultured Human Lymphocyte Clastogenicity Test: TX87014 dtd. Feb 1987. Batch CTD 6962/LM/TOX/1

Human lymphocytes exposed to BTS 58 726 at dose levels up to 35 µg/ml (maximum permitted by cytotoxicity) produced similar incidences of chromosome aberrations to that of negative controls.

BTS 59 482:

Ames Test: TX85101 dtd Dec 85. Batch TL2

BTS 59 482 did not show mutagenicity in the Ames test at concentrations up to 2500 µg/plate (maximum allowed by toxicity) in the presence and absence of S-9.

BTS 64 472

Ames Test: TX89011 (AT 0233) dtd Jan 1989. Batch TL2 Q.A.: Present (UK)

There was no consistent increase in numbers of revertant colonies for any indicator strain and thus, it was concluded that BTS 64 472 (up to 1250 μg/plate - max. tox. allowed) in the absence of any exogenous metabolic activation system, was not mutagenic in the Ames test.

Mammalian Cell, Chinese Hamster V79, Assay: TX89007 (VM 0017) dtd Jan 1989. Batch TL2. Q.A.: Present (UK).

There was no consistent increase in the number of 6-thioguanine-resistant mutants, and thus it was concluded that in the absence of any exogenous metabolic activation system BTS 64 472 (up to 200 μg/ml - max. tox. allowed) was not mutagenic at the HPRT locus in Chinese hamster V79 cells.

In Vitro Human Lymphocyte Chromosome Aberration Assay: TX89002 (LY 0021) dtd Jan 1989. Batch TL2. Q.A.: Present (UK).

No evidence of reproducible chromosome damage was seen at any concentration, thus it was concluded that BTS 64 472 (up to 150 μg/ml - max tox. allowed) in vitro in the absence of any exogenous metabolic activation was without clastogenic activity in this test.

Micronucleus Assay in Mice: TX89016 (MN 0015) dtd January 1989. Batch TL2. Q.A.: Present (UK).

Groups of 30M Olac MF-1 mice received single oral BTS 64 472 doses of 200, 400 or 800 mg/kg. Others were positive and negative controls. A number of mice from each group were killed at 24, 48, 72 hrs. (positive control at 24 hours). Femur bone-marrow smears were examined to determine the incidence of micronuclei in 1000 polychromatic erythrocytes per animal.

The incidence of micronuclei showed no statistically significant increases in BTS 64 472 groups - thus the drug was without in vivo clastogenicity in this test.

BTS 65 400

Ames Test: TX89009 (AT 0234) dtd Jan 1989. Batch TL1. Q.A.: Present(UK).

Replicate assays were conducted in the absence of any exogenous metabolic activation using concentrations up to 1250 μg/plate (Maximum allowed by BTS 65 400 toxicity).

The numbers of revertant colonies showed no consistent increase in numbers, thus the sponsor concluded that in the absence of any exogenous metabolic activation system BTS 65 400 was not mutagenic in the Ames test.

In Vitro Mammalian Cell (Chinese hamster V79) Mutation Assay: TX89008 (VM 0018) dtd January 1989. Batch TL1. Q.A.: Present (UK).

Replicate assays were carried out in the absence of any exogenous metabolic activation system using concentrations up to 200 μg/ml (maximum allowed by BTS 65 400 cytotoxicity).

The number of 6-thioguanine resistant mutants showed no consistent increase. Thus BTS 65 400 was not mutagenic at the HPRT locus in Chinese hamster V79 cells when tested in the absence of any exogenous metabolic activation system.

In Vitro Human Lymphocyte Chromosome Aberration Assay: TX89003 (LY 0022) dtd January 1989. Batch TL1. Q.A.: Present (UK).

Replicate assays were conducted in the absence of any exogenous metabolic activation system using concentrations up to 80 μg/ml (Maximum cytotoxicity permitted by BTS 65 400).

The incidence of chromosome aberrations (excluding gaps) of BTS 65 400 treated was comparable to negative controls. For the first assay,however, inclusion of gaps in the total aberrations for the 80 μg/ml group resulted in a statistically significant increase over control ($p = 0.0257$). The second

assay was without such a finding. In the absence of other types of chromosome damage the genotoxic significance of gap induction is uncertain. The sponsor believes the finding is probably related to cytotoxicity coupled with donor-sample variation.

No concentration showed any reproducible evidence of chromosome damage, thus BTS 65 400 was determined by the sponsor to be without in vitro clastogenic activity in this test.

Micronucleus Assay in Olac MF-1 Mice: TX89056 (MN 0016) dtd May 1989. Batch TL1. Q.A.: Present (UK).

In the first assay, groups of 30 male (no acute sex related differences) mice were given single oral doses of 12.5, 25 or 50 mg/kg BTS 65 400. Negative controls received 0.4% Cellosize and positive controls 75 mg/kg cyclophosphamide. Mice were killed at 24, 48 or 72 hours (24 hours for positive controls).

A second test was conducted with groups of 10 mice which were given single oral doses of 12.5, 25, 50 mg/kg BTS 65 400. There were 20 negative and 5 positive controls. All were killed 24 hours after dosing.

Femur bone-marrow smears were examined to determine the incidence of micronuclei in 1000 polychromatic erythrocytes per animal.

24 hours after 25 and 50 mg/kg BTS 65 400 treatment the first assay showed a small but statistically significant increase over the negative control value. It is reported that this negative control value was at the lower extreme of the previously observed range (0.10 to 0.44%) and the maximum value seen after BTS 65 400 (0.29% and 0.23%) only just exceeded the historical control mean of 0.23%. Results in the second assay were clearly negative.

The incidence of micronuclei of BTS 65 400 treated mice showed no reproducible, statistically significant increases. Thus according to the sponsor, BTS 65 400 was without in vivo clastogenicity when tested up to the maximum tolerated dose in this assay.

Additional Special Tests with BTS 64 472 and BTS 65 400:

BTS 64 472 - c-3-(1-amino-3-methylbutyl)-3-(4-chlorophenyl)-r-1-cyclobutanol fumarate, the aglycone of Metabolite 5 of BTS 54 524.

BTS 65 400 - 4-amino-4-[1-(4-chlorophenyl)cyclobutyl]-2-methylbutan-1-ol hydrochloride, the aglycone of Metabolite 6 of Product BTS 54 524.

BTS 64 472 and BTS 65 400 the reference salts of aglycones of metabolites 5 and 6 respectively. Study conducted to investigate plasma levels of BTS 64 472 and BTS 65 400 after a single oral dose of a mixture in rats in order to identify the oral mixture that would give an approximate 2:1 ratio of the compounds in plasma. [Metabolites 5 and 6 are the major human metabolites and are found in the ratio of 2:1, respectively, in human plasma, after an oral dose of sibutramine hydrochloride.]

Plasma Levels:

BTS 64 472 and BTS 65 400 - Preliminary Study to Investigate Plasma Levels in Rats. TX89106 (RA 0194) dtd October 1989. Batches: BTS 64 472 - TL2; BTS 65 400 - TL1. Q.A.: Present (UK).

Groups of 2M;2F Charles River CD rats received single oral doses of 20 mg of the mixture/kg. Each group received a mixture that was a 2:1, 2:5, 1:5 or 1:8 molar ratio of BTS 64 472:BTS 65 400 in Purified Water BP. Controls received vehicle alone. Animals were killed one hour after dosing and a blood sample was collected.

Mean total metabolite plasma ratios of 9.6:1, 6.8:1, 3.4:1 and 1.7:1, respectively were obtained.

It was decided that an oral dosing formulation of a 1:8 molar ratio of BTS 64 472:BTS 65 400 would be appropriate (give an approximate 2:1 ratio in plasma) for further testing.

A 1:8 Mixture of BTS 64 472 and BTS 65 400, Preliminary Short-Term Repeated Dose Range Finding Study in Rats: Report TX89107 (RC 0086) dtd October 1989. Batches: BTS 64 472 - TL2; BTS 65 400 - TL1. Q.A.: Present.

Groups of 3M;3F barrier-maintained Charles River CD rats were given 14 daily oral doses of the BTS 64 472 and BTS 65 400 mixture at doses of 0, 1, 3, 10 or 20 mg/kg - vol. 1 ml/100 g body weight. Plasma was analyzed for control and 20 mg/kg animals to determine the levels of the two metabolites pre- and post-hydrolysis (free aglycone and free + conjugated aglycones, respectively). Males were ca 40 days old and females ca 85 days old (ages at which dosing would normally begin in a fertility study).

In general signs were typical of an exaggerated pharmacological response which were consistent with the CNS stimulant effects that had been noted with sibutramine. Reduced weight gain was seen in some males which then recovered and gained more than controls. An overall weight loss was seen for some treated females in each treated group. Two high dose females showed marked losses during the first week.

20 mg/kg was determined to be a suitable high dose level for longer-term repeated-dose studies (a fertility study), but the 1 mg/kg dose was too high for a no-effect level.

A 1:8 Mixture of BTS 64 472 and BTS 65 400 - Preliminary Range Finding Study in Rats:

510-019. Report TX89116 dtd Nov 1989. Batches: (?) Q.A.: Present.

Range-finding study for a fertility study.

3M;3F per group were given 0, 0.5, 1, 10 and 20 mg/kg orally by gavage for 14 days followed by sacrifice and a blood sample for control and 20 mg/kg groups. Controls received deionized water.

Body weight gains for males were lower than controls at 10 and 20 mg/kg, but comparable to controls at 0.5 and 1 mg/kg. Female body weights were reduced for all groups during the first two days, and for 1, 10 and 20 mg/kg groups throughout the study.

All rats survived and there were no apparent treatment-related antemortem or necropsy findings.

Mean total plasma levels of respective metabolite entities were in a ratio of 1.1:1.

A 1:8 Mixture of BTS 64 472 and BTS 65 400 - Oral Study of Fertility in Rats. Report TX90054 dtd Jul 1990. Batches: (?) Q.A.: Present.

A mixture of BTS 64 472 and BTS 65 400 (1:8 mole ratio) was used to determine the effect on fertility and parturition of the F_0 generation, neonatal behavior, viability, growth, and reproductive capabilities of the F_1 generation.

Dose: 0, 0.5, 3.2 and 20 mg/kg p.o. by gavage Vol. 10 m/kg.
To males begining 60 days prior to mating and to females beginning 14 days prior to mating until sacrifice. Controls - ionized water.

No. Animals: 12 males and 24 females per group.
Charles River COBS CD rats.

Cesarean section of ca one-half per group was on day 20 - rest allowed to litter.
F_1 offspring were selected from each litter and evaluated for behavioral development. Selected F_1 males and females from the same treatment groups were mated to assess reproductive capabilities.

Results:
F_0 Group:

Survival was 100% except for one control F_0 female. Treated dams showed slight differences from the control group in reduced lactation period weight gain (not dose-related), reduced weekly food consumption (high dose females study week 1) also increased water consumption F_0 adults of all treated groups at various time periods. In general these differences were transient without representing significant parental toxicity.

F_0 male and female fertility and copulatory indices, pregnancy index, mean copulatory interval and mean gestation length were comparable to controls.

No treatment-related or statistically significant findings were seen in the mean number of viable fetuses, postimplantation loss, implantations, corpora lutea and fetal body weight or sex distribution for F_1 litters of Day 20 sacrificed dams.

F_1 and F_2 generations:

Fetal development including morphological examination, F_1 offspring survival, growth and behavior (including a number of indices) and F_1 adult general condition and reproductive capabilities did not appear to be adversely affected by the drug at any level. All control and treated groups were within normal limits with respect to neuropharmacological observations at weaning. There was a slight decrease in the percentage of weanlings able to perform the rotorod test for the high dose compared to the controls.

Incidental antemortem findings present in a few treated group litters (but not controls) included small size, sparse hair coat, paleness, missing distal tail and an abnormal gait.

At necropsy the majority of F_1 offspring were normal internally. Some instances of dead partially autolyzed pups were seen. Necropsy findings of one or two offspring in control and/or treated groups (considered incidental by the sponsor) included hydronephrosis, dark red kidney, white materia or clear fluid filled cysts in the kidney, distended ureter containing white material and/or fluid filled cysts in the spleen.

Reproductive parameters for the F_1 group and findings at C-section were comparable to controls.

The F_2 generation showed no apparent treatment-related differences in the incidence of external malformations and there were no external developmental variations presented.

1:8 Mixture of BTS 64 472 and BTS 65 400; Perinatal and Lactation Study in Rats: Report TX91081 dtd October 1991. Batches: (?) Q.A.: Present.

A mixture of BTS 64 472 and BTS 65 400 (1:8 mole ratio) was used to determine the effect on parturition and lactation of the F_0 generation, neonatal behavior, viability, growth, and reproductive capabilities of the F_1 generation.

Dose: 0, 0.5, 3.2 and 20 mg/kg p.o. as single daily doses by gavage
Gestation day 17 through day 20 of lactation
Vol. 10 m/kg. Controls ionized water.

No. Animals: 25 mated females per group
Charles River COBS CD rats.

All F_0 groups were allowed to deliver. F_1 pups were selected from each litter and evaluated for behavioral and developmental indices. Selected F_1 males and females from the same treatment groups but different litters were mated to assess their reproductive capabilities.

Cesarean sections were performed on the F_1 females on gestation day 20 and F_2 fetuses examined externally for teratology.

F_0 Groups:

One low dose sacrificed in extremis during delivery (red foci in stomach at autopsy). There were no treatment-related differences from controls in appearance, behavior or incidence of necropsy findings.

The high-dose level showed slight but statistically significant reductions (ca 5%) in maternal body weights compared to controls (mid-dose not significant). During lactation weight increase was less extensive than controls especially in mid and high dose groups. High dose mean food consumption was lower than controls during gestation. Water consumption was also lowest in the high dose group.

Reproductive capacities were not adversely affected. The gestation index and mean gestation length showed no treatment-related effects.

F_1 Groups:

Viability at day 0 and sex ratio were comparable to controls. At lactation days 0 and 4 there were slight decreases in high dose pup survival (100% mortality in one high dose litter by lactation day 1). Offspring survival was comparable to controls during the remainder of study. One high dose died at 18 weeks due to lung hemorrhage.

For the high dose group litters, survival indices were reduced and there were statistically significant reductions in body weights lactation days 0 and 4 compared to controls. High dose F_1 pups appeared small in size. Compared to controls high dose animals had slightly reduced body weights 6-20 weeks of age.

F_1 behavior/development indices were not adversely affected. Pinna detachment of the high dose on lactation day 2 was, however, reduced relative to concurrent controls, but was within that of historical controls. All treated and control offspring were within normal limits with respect to neuropharmacological observations at weaning. Learning and memory testing - passive avoidance showed a slight reduction from controls, more pronounced for the mid-dose females than high dose females.

Single instances of impaired limb function and a debilitated condition, a thread-like/missing tail and paleness were noted in low mid- and high dose litters.

Pups missing and presumed completely cannibalized were 2, 1, 6, 6 control through high dose. Due to partial cannibalization, complete necropsies could not be carried out on 1, 1, 3 pups in control, low and high doses.

One mid-dose had mandibular agnathia and an absent oral opening. Also in this group was a single instance of distended ureter. Findings in one or two rats in the low and mid-dose groups included hydronephrosis and cortical cysts in the kidney.

High dose F_1 maternal body weights showed statistically significant reductions (4-5%) compared to controls gestation days 0, 7, 17 and 20.

Male and female fertility indices, copulatory index and mean copulatory interval were comparable with controls.

Although not statistically significant there was a slight reduction in the mean number of implantations and mean number of viable F_2 fetuses in the high dose group. There were no dose-related differences in mean postimplantation loss, corpora lutea, fetal body weights, placental weight or sex distribution. However, there was a statistically significant increase in mean corpora lutea at the mid-dose but not at the high dose.

F_2 Fetuses:

There were no treatment-related differences in the incidence of external malformations and no external developmental variations.

Reprints: A number of reprints have been submitted which explore the drugs antidepressant and antiobesity properties.

Labeling: Needs Revision. See Recommendation below.

Summary - Tox/Reproduction/Mutagenicity:

Due to the voluminous amount of data and numerous studies presented in this NDA a "A narrative type Summary" of most pertinent studies is included. [See Table of Contents for location of full reports, also ADME etc.]

Single-dose studies have investigated the toxicity of sibutramine in mice, rats, dogs and cynomolgus monkeys. Repeat-dose studies were carried out for up to 3 months in mice, up to 6 months in rats and dogs and up to 1 year in cynomolgus monkeys. Fertility and general reproduction and peri- and post-natal studies were carried out in rats and teratogenicity studies were conducted in rats and rabbits. Carcinogenicity studies were conducted in mice and rats. A battery of short-term mutagenicity studies were presented.

Additionally, Sibutramine's metabolites were variously studied in acute, mutagenicity and reproduction studies in rats. Single dose studies also examined identified impurities and enantiomers of sibutramine.

Toxicology - Sibutramine:

[Excessive grooming leading to skin lesi ons was common in rodents.]

Acute study findings in **mice** included increased salivation, hyperactivity, excitability, aggression, stereotypic behavior, GI irritation, tremors and convulsions. Among similar findings some **rats** also showed evidence of CNS depression. **Dogs** also showed stereotypic head and mouth movements and convulsions at 40 mg/kg in the female. No treatment-related effects were seen on ECG or at autopsy. Findings in cynomolgus **monkeys** included pupillary dilation, excitability and increased activity with one case of visual disturbance. Biochemical changes were transient and hematology and ECG were unaffected.

Mouse:

3-Week: Doses up to 25.0 mg/kg produced dose-related pharmacological effects of increased activity, reduced body weight gain, increased food consumption, lower liver weight and disturbances of glycogen distribution within the liver lobules.

13-Week: Doses up to 50 mg/kg produced behavioral changes consistent with CNS stimulation. Body weight gain was slightly reduced in females and food consumption unaffected. Livers showed some glycogen loss and salivary glands cytoplasmic vacuolation in the acinar cells.

Rat:

2-Week: Doses up to 30 mg/kg showed reduced activity, tremorous movements, increased salivation, some reduced body weight and loss of condition. Other findings included reduced triglyceride, cholesterol, and total protein levels and in some increases in serum liver enzyme activity and bilirubin levels, with some slight hemoconcentration and increased in blood coagulation. GI irritation was present. Myelograms of some high dose females had an increase in myeloid cells.

4-Week: Up to 20 mg/kg doses showed some findings similar to the 2-week study as well as, serum creatine decreases, a slight decrease in urea levels of some females and decreased urine flow in both sexes. Some males had low liver weights.

26-Week: The study was conducted at doses up to 20 mg/kg with a 1 month recovery period. Behavioral changes consistent with CNS changes were evident (females>males) some persisting during the off-dose period. Both sexes showed a dose-related reduction in bodyweight gain during treatment. Food intake was reduced the first week. Ophthalmoscopy showed no treatment-related effects. Males had slightly higher Hb and PCV levels with increased MCV and MCH values early on and all treated groups had slightly increased myeloid:erythroid ratios terminally. Serum glucose, urea, potassium, creatinine, triglyceride and protein tended to be reduced, also lower Ca in males and lower bilirubin in females. A few had lower serum AST, ALT, AP and serum albumin with increases in gamma-globulin. Treated groups had higher urine specific gravity. Urine flow and SpG were slightly raised after recovery. Slightly elevated female salivary gland and kidney weights were normal after recovery. Uterine weight increases showed a slight recovery after the off-dose period. The higher incidence of centrilobular and midzonal hepatocyte vacuolation seen in males was apparent at a lesser extent after recovery. The higher incidence of macrophage aggregates in female lungs at 6 months was not increased after recovery.

Dogs:

Preliminary Studies: Studies up to 8-10 days and up to 20 mg/kg showed behavioral changes either subdued or excessively active with dilated pupils, salivation and stereotypic movements. Weight loss and reduced food consumption were evident, also occasional fecal blood loss. Slight hemoconcentration, increased serum AP, ALT activity and alterations in serum bilirubin and protein levels were noted. One 15 mg/kg male had low liver weight and hepatic glycogen loss - this dog also had myocardial hemorrhages of uncertain significance. In another study there was no apparent difference in toxic effects when sibutramine was given undiluted either in capsules or in solution by gavage.

4-Week: The study was carried out with up to 10 mg/kg in capsules. Behavioral changes, subdued or restless, and stereotypic movements were evident which lessened as treatment progressed. Salivation and emesis episodes were isolated. High dose dogs ate less readily during the first 2 weeks and lost weight slightly. High dose females had slightly increased platelet counts and two had marginally high AP activity. Liver weights showed some elevation in 3 dogs. No treatment-related effect was seen by ophthalmoscopy, ECG or urinalysis.

26-Week: Doses were up to 10 mg/kg followed by a 1 month recovery period. Stereotypic movements and overt signs seen previously including reduced food consumption and bodyweight loss were evident. Some dogs had periods when they became unresponsive to the presence of people. Slow or incomplete pupillary light reflexes and slightly low temperatures were evident. Urine volume was reduced in some that had eaten little or no food. Slightly higher cholesterol and AP were seen upon occasion in the high dose. No fecal occult blood was detected and no effects were noted on hematology, ophthalmoscopic or ECG exam. Some high dose livers appeared enlarged - no histopathological differences were noted.

Oculotoxicity: Carried out because sibutramine binds to melanin and accumulates in the uveal tract of the eye. 10 increasing to 15 mg/kg/day was given p.o. for 24 weeks. Behavioral, pharmacological, ECG, and autopsy findings were similar to other studies. Prolonged dilation of the pupils and inhibition of the pupillary light reflex were elicited by sibutramine, but there was no evidence of oculotoxicity.

Monkey:

13-Week: Doses were up to 10 mg/kg with a 6-week recovery period. Two deaths were not considered by the sponsor to be treatment-related. Initial weight loss was small and overall comparable with controls. There was a slightly increased incidence of vomiting. No effects were evident on food consumption, ocular changes or ECG. Hematology, clinical chemistry and urine appeared to be unaffected. At the end of treatment spleen weight tended to be lower in high dose monkeys.

[A preliminary study 10-30 mg/kg for up to 8 days showed CNS stimulation (30, 20 mg/kg stopped due to severity), hepatocellular fat vacuolation, but no effect on ECG or bone marrow. - A 7 day pilot study up to 15 mg/kg (also 5 days at 12.5 mg/kg) which produced hyperactivity, excitability and stereotyped behavior, bodyweight loss and reduced food consumption was used to set the 52 week study at doses up to 10 mg/kg.]

52-Week: Doses were up to 10 mg/kg. Behavior stimulation was observed in two high dose animals one a female also showed bodyweight loss and marked reduction in food consumption during the first 4-weeks. In general ECG, ophthalmoscopy, hematology (marginal significant increase in eosinophil count in males wk 25), blood chemistry (high dose females significant increase in triglycerides, potassium and calcium), urine analysis and post mortem findings (increased lung nodules control-high dose - males 0, 3, 0, 3; females 1, 2, 4, 1) did not show any apparent definite treatment-related changes.

Reproduction Studies: [Following a preliminary rat study (up to 20 mg/kg conducted in two similar phases) testing various parameters and types of cage floors, the sponsor concluded that 10 mg/kg was a suitable dose for the fertility and teratogenicity studies. Numbers were small and the two phases were not consistent - phase 2 showed evidence of embryotoxicity, fetotoxicity and retarded calcification - there were no similar effects at comparable dosages in phase 1.]

Fertility study: 0, 1, 3, 10 mg/kg (Males 60 and Females 14 days prior to mating until sacrifice)

Rat: Fertility and parturition of F_0 rats was not affected. Bodyweight gain, food and water intake were decreased at the higher doses (females high dose only). Two higher doses showed decreases in mean numbers of implantations and viable fetuses per litter with a slight increase in mean postimplantation loss (statistically significant at 10 mg/kg); also slight increase in number with retarded ossification of the skull. Neonatal bodyweight and survival of high dose pups were markedly affected. Cannibalism was extensive. Pup survival was unaffected at 1 and 3 mg/kg. High dose pup bodyweights were lower than controls during the lactation period. Mean bodyweights of pups of the low and mid-dose groups were also less than that of the control group but greater than that of the high dose group through lactation until 5-6 weeks of age; those kept for further testing approached or reached controls. F_1 progeny showed no apparent effects of behavior or reproductive performance (1 low dose and 2 mid-dose F_2 fetuses had microphthalmia).

Teratogenicity Studies:

Rat:

Rats were given 0, 1, 3 and 10 mg/kg days 7-17 of gestation. Bodyweight gain and food intake were decreased and hair was lost from limbs and ventral surface. There was no definite teratogenicity or increases in postimplantation loss indicating fetal death. Assessment of developmental toxicity showed no evidence of effect on the offspring and fetal growth in utero and postpartum were comparable with controls. However, a number of pups died during the lactation period, a number were autopsied and appeared normal, others were cannibalized. Behavioral and developmental values and F_1 offspring reproductive performance showed no disturbances. Hydronephrosis, white foci and cysts (male) of the lung were greater than that of controls.

Rabbit:

Dutch Belted: Preliminary studies in non-pregnant and pregnant Dutch Belted rabbits determined that 75 mg/kg would be appropriate for the highest dosage in the main study. However, 75 mg/kg proved too toxic due to marked CNS stimulatory and stereotypy effects as well as gastric lesions in the main study conducted with 0, 3, 15, 75 mg/kg. There was a slight increase in the incidence of intra-uterine death in the 75 mg/kg group (also reduced fetal wt. and reduction in skeletal calcification), also possibly at 15 mg/kg. Fetal viability of the high dose was significantly less than that for controls. At 75 mg/kg there was a slight increase in a particular syndrome manifest as a broad short snout, rounded pinnae, short tail, caudal vertebral anomalies and thickened limb bones.

Additional studies were carried out to show that this syndrome can occur spontaneously in this strain and that it appears to be mediated through males.

New Zealand White: [From a preliminary study the sponsor concluded that 50 mg/kg daily would be a suitable high dose.]

The main study was conducted with dosages of 0, 3, 12, 50 mg/kg Day 7-19. 50 mg/kg rabbits showed signs of CNS stimulation and stereotypic behavior and lost more weight only at the start of the dosing period - mean fetal weight was lower in this group (consistent with increased mean litter size). Cardiac anomalies included 1-low dose, 2 mid-dose, and 2 high dose - also one fetus from a single dose dam. Although there were none in controls this was reported as consistent with background for this strain and of genetic origin. The incidence of 13th rib and displacement of the pelvic girdle was increased. An additional study was conducted.

New Zealand White: Doses of 0, 12, 24 mg/kg day 6 to 18 post-coitum with sacrifice on Day 29. Pupillary dilation showed a dose related increased incidence. High dose food consumption was significantly lower days 6-12 of gestation and was associated with weight loss/reduced body weight gain during the first several days of dosing. The effect at 12 mg/kg was similar but not significant. The high dose showed a significant increase in the incidence of visceral and skeletal anomalies mainly an increased incidence of litters/fetuses with deviations in the origin of small arteries from the aorta and a significant increase in the incidence of litters/fetuses with ossified connection between the jugal and maxilla also fetuses with 13 ribs.

Peri- and Post-natal:

Rat: Doses were 0, 1, 3, 10 mg/kg Days 17-21 of lactation. [Due to excessive toxicity, 0.1 mg/kg was added and 10 mg/kg terminated during late gestation or early lactation.] F_0 toxicity at 10 mg/kg included deaths, reduced bodyweights and food and water, abnormal behavior (aggression, hyperactivity and cannibalization of apparently healthy offspring which contributed to marked reduction in F_1 survival). Significant offspring weight reductions were seen at 10 mg/kg. Similar findings were less severe at 3 and 1 mg/kg. 0.1 mg/kg was a no observable effect level (1 mg/kg for F_1 behavior and developmental parameters and 3 mg/kg for F_1 reproductive capacities).

Rat - Cross-fostering (lactation day 0): To determine and characterize effects on parturition and lactation as evidenced by neonatal viability and growth, 3 mg/kg was given Day 17 of gestation through Day 21 of lactation. Pups were cross-fostered on lactation day 0. Both dams and progeny were affected. Effects on dams resulted in retarded bodyweight gain in pups they were rearing, and direct effects on the progeny caused a higher incidence of neonatal mortality.

Carcinogenicity Studies:

Mouse: [Doses were reported chosen on results from 13-week and palatability studies.] Mice received 0, 0, 1.25, 5 or 20 mg/kg daily up to 104 weeks. Most high dose males had to be isolated from cagemates due to aggressive behavior or fighting injuries.

The number of palpable masses per animal was less than that of controls. High dose had lower bodyweights than controls (males ate slightly more from week 46 on). The overall incidence of benign and malignant tumors was not affected. A negative trend was evident for adenocarcinomas of the secretory stomach. There was a slightly increased incidence of serous cell vacuolation in high dose male salivary glands.

Trend test showed a marginally significant increased mortality in the high dose group compared to the other doses.

The positive linear trend in benign hemangioma in the uterus in female mice (2 high dose) is considered to be statistically significant.

Rat: [Doses were reported chosen on results from previous repeated-dose toxicity studies and the palatability study.]

Rats received 0, 0, 1, 3, 9 mg/kg daily for 104 weeks. Behavioral changes consistent with CNS stimulation included increased activity and vocalization in a few rats, increased aggression and piloerection. Females - increased cutaneous lesions, red, swollen limbs and digits. A dose related reduction in bodyweight was evident with associated reductions in food consumption. High dose males had a higher incidence of distended urinary bladder (leading to renal failure) and enlarged prostate (indicative of increased LH), and prostate inflammation.

The positive linear trend in benign interstitial-cell tumors in the testes of male rats is considered to be statistically significant.

Mutagenicity Tests: [concentrations = maximum allowed by cytotoxicity, etc.)

In vitro bacterial mutagenicity (Ames tests): **Negative** - 625 μg/plate in the presence of S-9 and 39 μg/ml in the absence of S-9.

In vitro mammalian cell mutation assay: **Negative** - 20 μg/ml in the absence of S-9, or up 80 μg/ml in the presence of S-9.

In vitro human lymphocyte clastogenicity assay: **Negative** - 250 μg/ml in the presence of S-9, 150 μg/ml in the absence of S-9.

In vivo micronucleus assay in mice: Two assays. Both assays gave marginal increases in the incidence of micronuclei in some mice at 250 mg/kg, the difference was not reproducible - it occurred at quite different sampling times i.e. 24 and 72 hours. There was no dose relationship; increased numbers of micronuclei occurred only in groups showing a marked reduction in hemopoiesis and/or excessive deaths (survival in some cases less than 50%). Sponsor concluded sibutramine was without in vivo clastogenicity.

Neurotoxic Assessment

On the basis of the studies reviewed (by Dr. J. F. Contrera), sibutramine does not possess the neurochemical properties generally associated with neurotoxicity in chemically related drugs.

Additional Special Studies:

Sibutramine is extensively metabolized in man and animals; however, the proportions of different metabolites vary with the species, thus their toxicity was investigated in a separate series of studies. A considerable number of studies (acute, reproduction, mutagenicity) were carried out in various species with enantiomers, metabolites (or mixtures of metabolites) or potential impurities of sibutramine. In general for most, qualitatively overt signs were similar to those with sibutramine but quantitatively pharmacological effects and toxicity varied. The possibility of a weak mutagen was shown in some tests with some compounds. [See main portion of review Special Studies beginning page 45.]

Findings of note included the following:

Quantitatively, toxicity of the (+) enantiomer was greater than that of sibutramine (50:50 racemate) in rats and monkeys and that of the (-) enantiomer was less.

The following compounds were found to be **very weak direct base-pair substitution mutagens in the Ames test** (small increases in numbers of TA 1535 revertant colonies): BTS 54 354 (reference salt of secondary amine of sibutramine); BTS 54 505 (reference salt of primary amine of sibutramine); BTS 58 726, a Stage III intermediate in sibutramine manufacturing and possible impurity (not detected at a limit of 0.05%). Acute toxicities of these three compounds in rats were similar to that of sibutramine.

The effects of a **mixture of BTS 64 472** (aglycone of Metabolite 5 of sibutramine) **and BTS 65 400** (aglycone of Metabolite 6 of sibutramine) in a molar ratio of 1:8 were determined in a **fertility and general reproduction rat** study at doses up to 20 mg/kg.

Transient slight differences from controls for F_0 included reduced weight gain during the lactation period (all treated dams), reduced food consumption (high dose dams) and increased water consumption (all treated groups). Additional parameters including F_1 and F_2 fetal development, survival, growth and behavior, general condition and reproductive capabilities of F_1 offspring did not appear to be adversely affected.

A **peri- and post-natal rat study** was also conducted with the 1:8 molar **mixture of BTS 64 472 and BTS 65 400** at dose up to 20 mg/kg. F_0 maternal body weights were slightly but statistically reduced. Both sexes of F_1 bodyweights were slightly reduced as were those during the gestation period. Reductions were seen in F_1 survival indices and in F_1 bodyweights (statistically significant) at lactation days 0 and 4 in the 20 mg/kg group. No apparent effects on reproductive capacities or neonatal behavior were present at 20 mg/kg.

F_2 fetuses showed no treatment-related differences in incidence of external malformations or external variations.

Separately neither compound was mutagenic in a battery of tests.

Comments and Conclusion:

[See also attached reviews by Rosloff, Contrera, Taneja]

Sibutramine is a substituted dimethylamine existing as a racemic mixture of two enantiomers. Originally developed as an antidepressant, it has shown potential as a weight-loss agent. [Both rodent and dog studies have shown dose-related weight loss (sometimes transient) and reduction in food intake.] The tertiary amine, Sibutramine, appears to be a non-selective neuronal reuptake inhibitor of biogenic amines, specifically serotonin and norepinephrine and to a lesser extent dopamine. It has been reported to down-regulate both beta-adrenergic receptor binding, as well as, the noradrenaline-stimulated adenylate cyclase system. Stimulant properties have been noted in animal studies, however, in a drug discrimination model trained rats did not recognize sibutramine as amphetamine-like.

The sponsor has proposed a dual mode of action by which sibutramine reduces body weight in animals; reduction of food intake through enhancement of satiety, and an increase in energy expenditure by induction of thermogenesis. Since sibutramine undergoes extensive first pass metabolism in the liver, such pharmacologic actions are mediated primarily by active metabolites. A dose-dependent reduction of food intake is seen in lean growing rats following acute administration of sibutramine or its active metabolites.

The drug and active metabolites also markedly and dose dependently reduce feeding in obese rats fed a high fat diet as well as in genetically obese Zucker rats. Such effects appear to be mediated centrally by enhancement of natural satiety responses via inhibition of 5-HT and norepinephrine reuptake resulting in increased activity at 5-HT 2A/2C and ß receptors respectively. No tolerance was observed and repeat dose dependent sustained reductions in body weight gain were seen in lean, growing genetically obese Zucker rats.

Sibutramine and a series of its metabolites have been found to have similar pharmacologic properties although the proportions vary from species to species. The actions of Sibutramine, which is rapidly absorbed from the GI tract, appear to be via its N-desmethyl secondary M1 and primary M2 amine metabolites. Following demethylation, two conjugated hydroxyl metabolites M5 and M6 are formed, both of these glucuronide conjugates appear to be relatively inactive.

Radiotracer studies in man have been reported to account for essentially all of the material in human plasma, thus attention was focused mainly on these metabolites. Metabolite 3 is the hydroxy analog of metabolite 2 and metabolite 4 is the trans-isomer of metabolite 5.
The parent compound, sibutramine, is a potent inhibitor of 5-HT and NE reuptake in vivo but not in vitro. However, M1 and M2 inhibit reuptake of these neurotransmitters both in vitro and in vivo (rats); they also show weak in vivo inhibition of dopamine reuptake.

Neither Sibutramine nor its metabolites M1 and M2 are 5-HT or dopamine releasing agents. Chronic administration of sibutramine to rats has not shown depletion of brain monoamines. Sibutramine and metabolites M1 and M2 are eliminated by hepatic metabolism (and biliary excretion) while renal excretion is the route of elimination for metabolites M5 and M6.

Metabolism was rapid giving metabolites 1 and 2 in the systemic circulation. Relative exposures were calculated from Cmax and AUC values of these metabolites. However, the HTD dose was based on 15 mg [the usual HTD not maximum (30 mg)] apparently in a 100 kg individual. [For obesity, toxicity calculations should be based on a 70 kg individual.] In the mouse metabolism was so fast that exposure to metabolite 1 could not be calculated. Exposure to metabolite 2 for the mouse, however, appeared to be similar (under these conditions) to that of man. Compared to man relative exposure of metabolite 1 appeared to be less than that for metabolite 2 which was greater for the rat than for rabbit and monkey (see p.6). Biotransformation of metabolite 2 produced metabolites 5 and 6 with relative exposure of metabolite 5 about 5 times higher in rabbit and monkey and metabolite 6 at least 10 times higher compared to man.

Effects of Sibutramine have been studied in mice rats, rabbits, guinea pigs, dogs and cynomolgus monkeys. Both pharmacologic and toxicologic studies have shown stimulant activity, excitability, aggression, and tremor.
ALT and AST elevations associated with hepatocyte change have also been seen. Although possible effects have been noted on the cardiovascular system in humans, electrocardiographic measurements showed no significant changes associated with treatment in the 52 week monkey study. General pharmacology would tend to indicate slight pressor effects. Sibutramine-related material tends to bind reversibly to melanin-containing tissue (as do other organic amines), however, the 24 week study in dogs with emphasis on the eyes showed no evidence of oculotoxicity.

Reproduction studies have shown marked maternal toxicity. There were no effects on fertility and no specific evidence of teratogenicity; however, at the 10 mg/kg dose there was a higher incidence of perinatal mortality in rats.
The rat teratology study showed a larger number of F_1 from treated groups with hydronephrosis of the kidney at necropsy which appear to be treatment related.

Although Dutch belted rabbits had an increased incidence of malformations, these appear to be related to a syndrome of malformations characteristic of the strain used. Thus, these studies are not suitable for an evaluation of teratogenic effects.

Studies were then carried out in New Zealand White rabbits which at 75 mg/kg showed behavioral changes, overt signs and body weight losses to be proportionately greater than that seen in the Dutch Belted rabbits. 50 mg/kg which also caused signs of CNS stimulation and stereotyped behavior was chosen as a suitable high dose (doses 0, 3, 12, 50 mg/kg). Although not seen in controls, 1-2 cardiac anomalies (mainly stenosis or atresia of pulmonary trunk or valve) were produced in each treated group. The incidence was slightly greater than that of the background incidence in this strain of rabbit. The increased incidence of the developmental variant pelvic girdle shift was only a slight deviation from normal. Another teratology study was carried out at 0, 12, 24 mg/kg; anomalies were different than in previous studies being increases in the incidence of litters/fetuses with deviations in the origin of small arteries from the aorta and ossified connection between the jugal and maxilla and 13th ribs. There appeared to be no specific teratology and the incidence of varied anomalies was not consistent among the different studies.

10 mg/kg produced excessive toxicity in the rat perinatal study which included a marked increase in the mean number of stillborn and live young at lactation day 0. Decreased survival of F_1 offspring was also observed at 3.0 mg/kg. Increased cannibalism was evident apparently including that of healthy offspring. Neglect, CNS stimulation and stereotypic behavior may also have contributed to the early demise of these pups. One 3.0 mg/kg pup had stenosis of the aortic arch. 1.0 mg/kg malformations included situs inversus in one weanling and interventricular septal defect in twolittermates. The relevance of the findings of stenosis and septal defects in rat and rabbit are unknown. (Use of the drug is not recommended in pregnant women.)

A cross-fostering perinatal and lactation study was carried out in rats to explore this situation. Although not statistically significant, the index for pup survival from cross-fostering to lactation day 4 was lower for treated pups cross-fostered to control dams compared to control pups cross-fostered to treated dams. After day 4, the number of deaths was comparable between control pups cross-fostered to treated dams and treated pups cross-fostered to control dams. Thus, it appears that the drug affects both mother and pups born to treated mothers. Placental transfer is evident in both rat and rabbit. Labeling recommends that MERIDIA not be used in pregnant women or in nursing mothers.

Carcinogenicity studies in general showed no effects on tumor production in male mice or in female rats. However, the positive linear trend in benign hemangioma in the uterus of 2 high dose female mice and in benign interstitial-cell tumors in the testes of male rats are considered to be statistically significant. The trend test showed a marginally significant increased mortality in high dose mice compared to the other doses. Renal failure, the most frequent cause of death in this group, was considered to be associated with urinary stasis and bladder obstruction.

The relevance of the finding of benign hemangiomas in the uterus of two high-dose (only) rats is unknown.

The increased incidence of benign interstitial-cell (Leydig-cell) tumors of the testes (1, 5, 6, 6, control-high dose - significant positive trend: p=0.013) was higher than that of concurrent controls, but was reported to be within background range for the strain at Boots. They indicate that the tumors usually appear in old rats and are treatment related rather than being dose-related; also, that limited work reported on the pathogenesis of interstitial tumors suggests that important features are reduced plasma testosterone concentration and consequent increased pituitary-LH secretion, possibly together with suppression of the natural increase in plasma prolactin concentration.

From measurements in the rat carcinogenicity study they concluded that increased LH and FSH levels might at least be part of the mechanism of interstitial-cell hyperplasia and adenoma associated with long-term sibutramine treatment of male rats.

The incidence of Leydig-cell tumors is quite variable and often frequent in male rats, but very rare in humans. Such tumors have been associated with a variety of compounds in rats none of which has been associated with testicular tumors in man. The problem of Leydig-cell tumors does not appear to affect mice. Thus, it is possible that the finding of such tumors in rats may be species specific and not relevant for man.

Mutagenicity studies with Sibutramine showed no evidence of genotoxicity in the Ames test, in vitro mammalian cell mutation assay, in vitro human lymphocyte clastogenicity, or in vivo clastogenicity mouse micronucleus assay.

The reference salts of the primary and secondary amines of sibutramine and an intermediate in manufacture and possible impurity were found to be very weak direct base-pair substitution mutagens in the Ames test. The significance of the findings of these small increases in numbers of TA 1535 revertant colonies is unknown. [Tests in mammalian cells in vitro and mammalian systems in vivo were negative.] Acute toxicities of these three compounds in rats were similar to that of sibutramine.

According to CDER Expert Reviewer for Pharmaceutical Neurotoxicology, Dr. Joseph F. Contrera (review dtd. 13 Jun 96, attached), on the basis of the studies reviewed, sibutramine does not possess the neurochemical properties generally associated with neurotoxicity in chemically related drugs.

Sibutramine is extensively metabolized in man and animals; however, the proportions of different metabolites vary with the species, thus their toxicity was investigated in a separate series of studies. A "considerable number" of studies (acute, reproduction, mutagenicity) were carried out in various species with enantiomers, metabolites (or mixtures of metabolites) or potential impurities of sibutramine (See Special Studies, this review p. 45.) In general for most, qualitatively overt signs were similar to those with sibutramine but quantitatively pharmacological effects and toxicity varied. There were reductions in F_1 neonatal viability and growth at high doses in the peri- and post-natal study with a mixture of two of the metabolites, but in general no effects on reproductive capabilities of the dams nor on neonatal behavior.

The lack of some Quality Assurance statements was brought to the attention of the sponsor by phone 15,16 Jul 96. This was followed by submission of a large volume (1,5 Aug 96) containing GLP and QA statements available for preclinical study reports. A few of the studies (mostly mid-1980's), although having signed QA statements, have no dates of QA inspection. A statement indicating that study reporting had varied during certain time periods was provided. From the information in the original submission it appeared that the drug had been received and the studies carried out. This situation, however, was brought to the attention of DSI and discussed with Dr. Earl Butler, HFD-345 (any decision as to future inspections will be made by DSI).

Recommendation:

Pharmacology recommends approval of MERIDIA (sibutramine hydrochloride) for the long-term treatment of obesity in conjunction with diet and exercise as part of a weight management program. However, Labeling needs revision.

re Labeling:
The Contraindications section of the Labeling states: MERIDIA should not be given to pregnant women or nursing mothers. Unless supported by adequate studies in humans, this may not be the appropriate place for such a statement; this section of the labeling, however, is under the purview of the Medical Officer. This recommendation can be included under the Pregnancy and Nursing Mothers sections.

To be Conveyed to the Sponsor:
The following information concerning labeling should be conveyed to the sponsor.

Labeling needs revision according to 21 CFR 201.57 which includes the following:

1) The basis for determining the multiple of the maximum human dose should be stated in the labeling. When plasma drug levels are available, human exposure should be expressed in terms of multiples of the AUC observed in preclinical studies. [Please provide calculations, but do not include them in the labeling.]

2) Carcinogenicity section: The sentence regarding benign tumors of testicular interstitial cells should be changed to read: "In male rats, there was a higher incidence of benign tumors of the testicular interstitial cells; such tumors commonly seen in rats are hormonally mediated." The following sentence should be added: Relevance of these tumors to humans is not known."

3) The fertility statement should be placed in the section entitled Carcinogenesis, mutagenesis, impairment of fertility.

4) The Pregnancy section should include the following sentence: Meridia is not recommended for use in pregnant women.

NOTE: [Do not convey to sponsor.]
The Nursing Mothers section contains a not recommended statement.

A statistically significant positive linear trend was found for (2 only) benign hemangiomas in the uterus of the mouse carcinogenicity study high dose group. Considering the low number and this finding being only a trend, it will not have to be included in the labeling.

The sponsor has recently changed the maximum HTD from 30 to 20 mg.

/

David H. Hertig
Pharmacologist

cc:
Original NDA 20-632;
HFD-24 JDeGeorge; HFD-400 JContrera
HFD-345 HFD-510 NDA 20-632; IND 27,264
HFD-510 RSteigerwalt; MHess; DHertig

10/4/96

NDA 20-632

Date: June 13, 1996

Assessment of the Neurotoxic Potential of Meridia (sibutramine).

Pharmaceutical: Meridia capsules; sibutramine hydrochloride monohydrate; BTS 54 524.

Sponsor: Knoll Pharmaceutical Company
North Mount Olive, New Jersey 07828

Review Division: Division of Endocrine and Metabolic Drug Products.

Reviewer:

Dr. Joseph F. Contrera
FDA/CDER Office of Testing and Research, HFD-900
Office of Pharmaceutical Sciences
CDER Expert Reviewer for Pharmaceutical Neurotoxicology

Previous Pharmacology/Toxicology Reviews:

Division of Neuropharmacological Drug Products (HFD-120) pharmacologist reviews of January 22, 1986, February 19, 1986 and October 12, 1989. Neuropharmacology, ADME and 4 week toxicity studies in rats and dogs were evaluated by Dr. Barry Rosloff.

Submissions Reviewed:

NDA 20-632 volumes 1.40-1.45 and submission of April 8, 1996.

TABLE OF CONTENTS

EVALUATION

Sibutramine: Effects on Brain Neurotransmitter Levels.

1. Single Dose Studies.

Oral administration of 10 mg/kg sibutramine to mice and rats did not result in any significant alteration in whole brain dopamine or serotonin levels measured 2 hours and 24 hours after treatment (Table 1, 2). An evaluation of alterations in dopamine and serotonin levels in the striatum and hippocampus was carried out under the same experimental conditions in rats and no alterations in dopamine and serotonin levels were observed (Table 3). Whole brain 5HT depletion in rats was reported after a single 7.5 mg/kg dose of d-fenfluramine which persisted for up to 168 days after cessation of treatment (Garratini, et al., 1986).

2. Multiple Dose Studies.

Rats were treated daily with 3 mg/kg/day of oral sibutramine and brain striatal and hippocampal dopamine and serotonin were measured at 2 hours, 24 hours, 7 days, 14 days and 21 days of treatment. Animals were sacrificed 2 hours after the last drug treatment. Sibutramine had minimal effects on dopamine and serotonin levels and produced alterations in dopamine and serotonin metabolites consistent with alterations in neurotransmitter turnover consistent with the expected action of sibutramine on neurotransmitter reuptake processes (Table 4). A sibutramine dose of 3 mg/kg is approximately the ED50 for food intake inhibition in rats.

Seventy two day treatment: Sibutramine was administered to rats once daily at 3 mg/kg/day (oral) for 72 days. Animals were sacrificed 2 hours after the last drug treatment. No significant changes in brain norepinephrine, dopamine or serotonin levels were observed (Table 5).

Sibutramine: Effects on Neurotransmitter Release and Reuptake.

An in vivo study compared alterations in the extracellular level of serotonin in rat hypothalamus after single i.p. doses of sibutramine, fluoxetine and d-fenfluramine at 1, 3 and 10 mg/kg. Sibutramine and fluoxetine produced relatively small increases in extracellular serotonin mainly at the highest dose evaluated. In comparison, d-fenfluramine produced a rapid, highly significant increase in extracellular serotonin that was proportional to dose (Fig. 1).

In an in vitro study employing rat brain slices, sibutramine or its active metabolites did not stimulate the release of tritiated dopamine, norepinephrine or serotonin. In comparison, d-fenfluramine stimulated the release of serotonin and d-amphetamine stimulated the release of all three neurotransmitters to varying degrees (Table 6).

The two major metabolites of sibutramine, the secondary amine metabolite 1 (BTS 54 354) and the primary amine metabolite 2 (BTS 54 505) are more potent uptake inhibitors of norepinephrine, dopamine and serotonin than sibutramine (Table 7). These major sibutramine metabolites are produced in animals and humans and appear to be responsible for most of the pharmacological actions of sibutramine.

As was observed with fluoxetine, sibutramine pretreatment of rats (10 mg/kg i.p.) attenuates d-fenfluramine induced release of serotonin in the hypothalamus (Fig. 2). This demonstrates that sibutramine does not stimulate the release of serotonin in vivo and sibutramine blocks serotonin reuptake which is necessary for the serotonin releasing action of d-fenfluramine.

Sibutramine: Effects on Serotonin High Affinity Reuptake Sites.

Regional brain serotonin reuptake sites were evaluated employing tritiated paroxetine binding. Sibutramine (9 mg/kg) and d-fenfluramine (3 mg/kg) were administered orally twice daily for 4 days followed by a 14 day recovery period. Sibutramine did not produce any significant change in the number of tritiated paroxetine labelled serotonin binding sites in various regions of rat brain. In contrast d-fenfluramine treatment resulted in a highly significant reduction in the population of tritiated paroxetine labelled serotonin binding sites in the frontal cortex, hippocampus and hypothalamus (Fig. 3).

Sibutramine: Effects on Serotonin 5HT-2 Receptors.

Rats were treated with 10 mg/kg p.o. of sibutramine, fluoxetine, d-fenfluramine and d-amphetamine once daily for 14 days. 5HT-2 binding sites were evaluated in frontal cortex membrane preparations employing tritiated ketanserin. Sibutramine had no effect on 5HT-2 binding sites. Of all the drugs tested only d-fenfluramine produced highly significant reduction in the number of 5HT-2 binding sites (Table 8).

CONCLUSIONS

The general definition of neurotoxicity presented in 40 CFR Ch.1 (7-1-91) pg.546.

> An adverse effect on the structure or function of the central and/or peripheral nervous system related to exposure to a chemical substance.

For drug products, benefit/risk considerations are critical in evaluating the significance of an "adverse effect".

In this review, the capacity of a compound to significantly alter brain neurotransmitter biomarkers was used as a preliminary indicator of neurotoxic potential. These biomarkers include: a) a reduction in brain 5HT, DA or NE levels with particular attention to effects that persist for extended periods (weeks-months) after cessation of drug treatment, b) a reduction in the number of 5HT uptake sites in the brain, c) evidence of rapid, extensive drug related release of neurotransmitter. Depletion of brain neurotransmitter that persists after cessation of drug treatment may be associated with axonal degeneration in animals. Although alterations in these biomarkers alone are not sufficient proof of neurotoxicity, reference neurotoxins such as 5,7-dihydroxytryptamine (5,7 DHT), p-chloroamphetamine (PCA), methylenedioxymethamphetamine (MDMA) and DSP-4 which interact with brain 5HT, DA and NE neurons respectively, profoundly affect these biomarkers (Moliver et al. 1990; Fritschy and Grazanna, 1992).

In the studies reviewed, sibutramine did not produce depletion in brain norepinephrine, dopamine or serotonin after acute or chronic administration in rodents at doses equal to or exceeding approximately 3 times the ED50 for food intake suppression.

Sibutramine appears to be a more effective inhibitor of serotonin and norepinephrine reuptake than d-fenfluramine but does not stimulate the release of either norepinephrine, serotonin or dopamine. In an in vitro study employing rat brain slices, sibutramine or its active metabolites did not stimulate the release

of tritiated dopamine, norepinephrine or serotonin. In comparison, d-fenfluramine stimulated the release of serotonin and d-amphetamine stimulated the release of all three neurotransmitters to varying degrees.

As was observed with fluoxetine, sibutramine pretreatment of rats (10 mg/kg i.p.) attenuates the d-fenfluramine induced release of serotonin in the hypothalamus. This demonstrates that sibutramine does not stimulate the release of serotonin in vivo and also blocks the serotonin reuptake which is necessary for the serotonin releasing action of d-fenfluramine. The drug related release of neurotransmitter is a mechanism that has been linked to the axonal degeneration and neurotoxicity produced by drugs such as amphetamine and MDMA. The release of serotonin is also associated with long lasting depletion of brain serotonin induced by d-fenfluramine (Gobbi et al., 1993).

Sibutramine does not alter the number of 5HT uptake sites of the brain. Sibutramine (9 mg/kg) and d-fenfluramine (3 mg/kg) were administered orally twice daily for 4 days followed by a 14 day recovery period. Sibutramine did not produce any significant change in the number of tritiated paroxetine labelled serotonin binding sites in various regions of rat brain. Sibutramine treatment of rats (10 mg/kg p.o.) for 14 days had no effect on the number of 5HT-2 receptors which can be used as an indicator of up or down regulation of this receptor or axonal degeneration. The reduction in the number of serotonin uptake sites is correlated with the long term depletion of 5HT induced by d-fenfluramine (Appel et al., 1989).

A reduction in the number of axonal uptake sites has been correlated with neurodegeneration associated with neurotoxins that act upon the serotonergic, dopaminergic or noradrenergic system, and to disease related neuropathology. A reduction in dopamine uptake binding sites is associated with MPTP, MDMA and 6-hydroxydopamine neurotoxicity as well as with disorders such as Parkinsons disease and progressive supranuclear palsy (Hitri et al., 1994). A reduction in 5HT uptake binding sites in cortical areas also occurs in Alzheimer type dementia (Palmer, et al., 1987).

RECOMMENDATION

On the basis of the studies reviewed, sibutramine does not possess the neurochemical properties generally associated with neurotoxicity in chemically related drugs.

Joseph F. Contrera, Ph.D.

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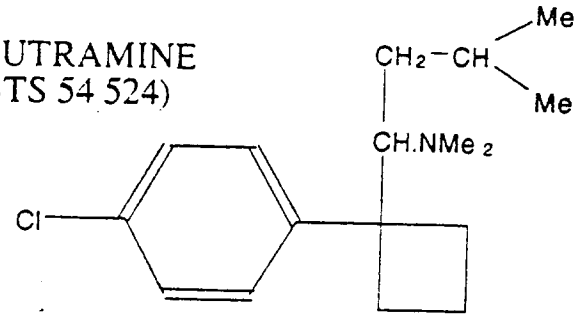
APPENDIX

NDA 20-632 Date: June 13, 1996

Assessment of the Neurotoxic Potential of Meridia (sibutramine).

THE STRUCTURE OF SIBUTRAMINE AND ITS METABOLITES

SIBUTRAMINE
(BTS 54 524)



Sibutramine and its major active metabolites, Metabolite 1 and Metabolite 2, are arylcyclobutylalkylamines.

Sibutramine is a tertiary amine. When administered to animals and man, it is rapidly demethylated to form Metabolite 1 and Metabolite 2.

The carbon atom to which the amine group is attached is a chiral centre for sibutramine, Metabolite 1 and Metabolite 2.

TABLE 1.

Effects of oral administration of BTS 54 524 (10 mg kg^{-1}), dothiepin (100 mg kg^{-1}) and citalopram (20 mg kg^{-1}) on mouse whole brain levels of monomines and metabolites (NA not determined).

Experiment	DA	DOPAC	HVA	5-HT	5-HIAA
			ng/g wet wt.		
Saline (2h)	1163 ± 30	74 ± 5	161 ± 6	581 ± 16	219 ± 11
BTS 54 524 (2h)	1206 ± 26	54 ± 3**	183 ± 12	635 ± 13	209 ± 9
Dothiepin (2h)	1190 ± 37	87 ± 7	180 ± 12	597 ± 16	202 ± 10
Citalopram (2h)	1145 ± 44	94 ± 8	175 ± 11	569 ± 23	132 ± 8***
Iprindole (2h)	1241 ± 19	99 ± 6	180 ± 6	626 ± 26	279 ± 16*
Saline (24h)	1267 ± 44	64 ± 6	151 ± 5	585 ± 4	206 ± 10
BTS 54 524 (24h)	1158 ± 34	67 ± 4	166 ± 8	577 ± 8	206 ± 5
Dothiepin (24h)	1231 ± 45	65 ± 6	161 ± 8	592 ± 5	236 ± 8
Citalopram (24h)	1154 ± 52	56 ± 6	141 ± 7	587 ± 14	205 ± 7
Iprindole (24h)	1181 ± 24	54 ± 6	148 ± 7	609 ± 19	247 ± 11*

$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to saline controls.

TABLE 2.

Effects of oral administration of BIS 54 524 (10 mg kg^{-1}), dothiepin (100 mg kg^{-1}) and citalopram (20 mg kg^{-1}) on rat whole brain levels of monomines and metabolites (NA not determined).

Experiment	DA	DOPAC	HVA	5-HT	5-HIAA
	ng/g wet wt.				
Saline	880 ± 48	62 ± 5	96 ± 5	437 ± 33	279 ± 15
BIS 54 524 (2h)	821 ± 61	36 ± 4**	77 ± 5*	365 ± 30	170 ± 20**
Dothiepin (2h)	913 ± 27	53 ± 3	82 ± 2	431 ± 5	232 ± 17
Citalopram (2h)	990 ± 17	67 ± 4	109 ± 5	471 ± 10	173 ± 14***
Iprindole (2h)	1019 ± 23	65 ± 3	100 ± 4	446 ± 14	268 ± 14
Saline (24h)	886 ± 19	64 ± 3	92 ± 4	455 ± 22	270 ± 10
BIS 54 524 (24h)	890 ± 33	51 ± 4*	96 ± 9	445 ± 22	237 ± 8*
Dothiepin (24h)	964 ± 22*	56 ± 1*	98 ± 6	470 ± 31	271 ± 26
Citalopram (24h)	979 ± 27*	71 ± 3	95 ± 5	520 ± 31	252 ± 7
Iprindole (24h)	965 ± 14	66 ± 2	82 ± 3	538 ± 35	313 ± 9

*p<0.05, **p<0.01, ***p<0.001 compared to saline controls.

TABLE 3.

Effects of oral administration of BTS 54 524 (10 mg kg^{-1}), dothiepin (100 mg kg^{-1}) and citalopram (20 mg kg^{-1}) on rat striatal DA, DOPAC, HVA, and rat hippocampal 5-HT and 5-HIAA

Experiment	STRIATAL			HIPPOCAMPAL	
	DA	DOPAC	HVA	5-HT	5-HIAA
	ng/g wet wt.				
Saline (2h)	9108 ± 329	921 ± 56	755 ± 32	314 ± 20	263 ± 15
BTS 54 524 (2h)	9041 ± 363	601 ± 17***	595 ± 17**	339 ± 14	247 ± 10
Dothiepin (2h)	9005 ± 494	747 ± 62	586 ± 44*	333 ± 12	256 ± 10
Saline (2h)	9036 ± 502	673 ± 56	715 ± 57	318 ± 18	275 ± 8
Citalopram (2h)	10089 ± 285	915 ± 24**	842 ± 34	326 ± 28	198 ± 17**
Iprindole (2h)	10336 ± 265	805 ± 43	714 ± 49	282 ± 9	354 ± 20
Saline (24h)	9079 ± 333	872 ± 83	745 ± 43	295 ± 22	249 ± 8
BTS 54 524 (24h)	8606 ± 411	697 ± 54	782 ± 77	327 ± 15	216 ± 12
Dothiepin (24h)	9812 ± 165	876 ± 40	780 ± 40	291 ± 19	282 ± 23
Saline (24h)	9201 ± 436	852 ± 23	830 ± 22	327 ± 9	349 ± 27
Citalopram (24h)	8796 ± 474	869 ± 67	670 ± 64	325 ± 23	222 ± 11**
Iprindole (24h)	10751 ± 775	929 ± 95	850 ± 38	332 ± 19	353 ± 22

*p<0.05, **p<0.01, ***p<0.001 compared to saline controls.

TABLE 4.

The effects of a single oral dose and consecutive daily doses of BTS 54 524 (3 mg kg^{-1}) on rat striatal DA, DOPAC, HVA and rat hippocampal 5-HT and 5-HIAA levels.

Experiment	STRIATAL			HIPPOCAMPAL	
	DA	DOPAC	HVA	5-HT	5-HIAA
	ng/g wet wt.				
Control (2h)	13148 ± 493	1181 ± 39	934 ± 37	175 ± 9	179 ± 12
BTS 54 524 (2h)	11930 ± 433	935 ± 33***	834 ± 42	140 ± 8*	135 ± 10*
Control (3 day)	10341 ± 590	1120 ± 76	640 ± 33	159 ± 7	171 ± 5
BTS 54 524 (3 day)	9833 ± 315	1002 ± 45	840 ± 55**	165 ± 9	173 ± 10
Control (7 day)	9773 ± 364	839 ± 49	774 ± 51	129 ± 7	151 ± 8
BTS 54 524 (7 day)	9947 ± 391	887 ± 55	829 ± 62	125 ± 2	184 ± 8*
Control (14 day)	11906 ± 557	967 ± 28	674 ± 34	153 ± 10	125 ± 9
BTS 54 524 (14 day)	10197 ± 387*	880 ± 38	699 ± 42	154 ± 9	109 ± 5
Control (21 day)	11814 ± 454	1201 ± 40	801 ± 42	150 ± 14	167 ± 15
BTS 54 524 (21 day)	13582 ± 563*	1337 ± 75	1702 ± 81***	177 ± 14	161 ± 12

*p<0.05, **p<0.01, ***p<0.001 compared to control values

TABLE 5.

The effects of once-daily oral administraion of BTS 54 524 (3 mg kg^{-1}) for 72 days on rat whole brain levels of monoamines and metabolites.

Experiment	NA	DA	DOPAC	HVA	5-HT	5-HIAA
	ng/g wet wt.					
Control	263 ± 13	754 ± 45	61 ± 4	96 ± 5	283 ± 20	242 ± 23
BTS 54 524	258 ± 21	778 ± 46	56 ± 5	109 ± 9	258 ± 27	159 ± 16*

*p<0.05 compared to control values

TABLE 6.

EFFECT OF SIBUTRAMINE AND ITS METABOLITES ON [3H]NE, [3H]5-HT AND [3H]DA RELEASE FROM RAT BRAIN SLICES IN VITRO

	Uptake Ki (nM)	Release Concentration (nM)		
		100	1000	10,000
NE				
Sibutramine	283	±	±	±
Metabolite 1	2.7	±	±	±
Metabolite 2	4.9	±	±	±
d-Amphetamine	45	57	135	162
d-Fenfluramine	260	±	±	82
5-HT				
Sibutramine	3131	±	±	±
Metabolite 1	18	±	±	±
Metabolite 2	26	±	±	±
d-Amphetamine	1441	±	±	136
d-Fenfluramine	279	±	64	282
DA				
Sibutramine	2309	±	±	±
Metabolite 1	24	±	±	±
Metabolite 2	31	±	±	±
d-Amphetamine	132	56	122	133
d-Fenfluramine	6227	ND	ND	ND

± = no significant effect, 56 - 282 (%) significant elevation, ND = not determined

Heal et al., Psychopharmacology 107 (1992) 303-309. Heal et al., Br. J. Pharmac. 117 (1996) 325P.

Sibutramine and Metabolites 1 and 2 do not stimulate the release of [^{3}H]norepinephrine, [^{3}H]5-HT or [^{3}H]dopamine from brain slices in vitro at concentrations up to 10,000 nM. For Metabolites 1 and 2, which are the predominant moieties found in animals and man, these concentrations are 400 to 4000-fold higher than their Ki values for inhibition of [^{3}H]monoamine uptake.

d-Amphetamine significantly stimulates [^{3}H]norepinephrine and [^{3}H]dopamine release at 100 nM. There is no separation between the activity of d-amphetamine as a releaser and a reuptake inhibitor of [^{3}H]norepinephrine and [^{3}H]dopamine.

d-Fenfluramine significantly stimulates [^{3}H]5-HT release at 1000 nM. There is only a 3-fold separation between the actions of d-fenfluramine as a releaser and as a reuptake inhibitor of [^{3}H]5-HT.

TABLE 7.

POTENCIES OF SIBUTRAMINE, ITS METABOLITES AND REFERENCE COMPOUNDS AS MONOAMINE REUPTAKE INHIBITORS IN RAT BRAIN

	Potency to inhibit monoamine reuptake (Ki; nM)		
	NE	5-HT	DA
	Rat brain tissue		
Sibutramine	283	3131	2309
Metabolite 1	2.7	18	24
Metabolite 2	4.9	26	31
Desipramine	1.7	200	4853
Fluoxetine	320	11	2025
d-Amphetamine	45	1441	132
d-Fenfluramine	260	279	6227

Cheetham et. al., Neuropharmacology 32 (1993) 737-743.
Cheetham et. al., Neuropharmacology 35 (1996) 63-70.

The in vitro reuptake profile of sibutramine and its major active metabolites have also been compared with those of the reference monoamine reuptake inhibitors, desipramine and fluoxetine, and also with those of d-amphetamine and d-fenfluramine.

As in vitro inhibitors of monoamine reuptake, Metabolite 1 and Metabolite 2 are approximately as potent against norepinephrine as the selective norepinephrine reuptake inhibitor, desipramine, and against 5-HT as the selective 5-HT reuptake inhibitor (SSRI), fluoxetine.

The monoamine reuptake inhibition profiles of Metabolite 1 and Metabolite 2 are markedly different to those of d-amphetamine and d-fenfluramine. d-Amphetamine is a moderately potent inhibitor of norepinephrine reuptake and weak inhibitor of 5-HT reuptake. d-Fenfluramine is a weak inhibitor of both norepinephrine and 5-HT reuptake.

TABLE 8.

Effects of repeated administration of sibutramine and other weight modifying agents on rat cortical 5-HT_2 receptors

Drug	Bmax (fmol/mg protein)	Kd (nM)
Vehicle	433 ± 16	0.51 ± 0.02
Sibutramine	442 ± 13	0.47 ± 0.01
Fluoxetine	461 ± 12	0.53 ± 0.02
d-Fenfluramine	175 ± 5***	0.60 ± 0.01**
d-Amphetamine	444 ± 15	0.50 ± 0.01

Groups of rats were administered drugs at a dose of 10 mg/kg po or vehicle (distilled water) once daily for 14 days.

5-HT_2 receptor binding was measured in rat frontal cortex 24h after the final treatment.

re given as mean ± SE (n = 10 drug-treated groups; n = 20 vehicle-treated group).

tly different from vehicle-treated control. ** p<0.01, *** p<0.001.

Results a

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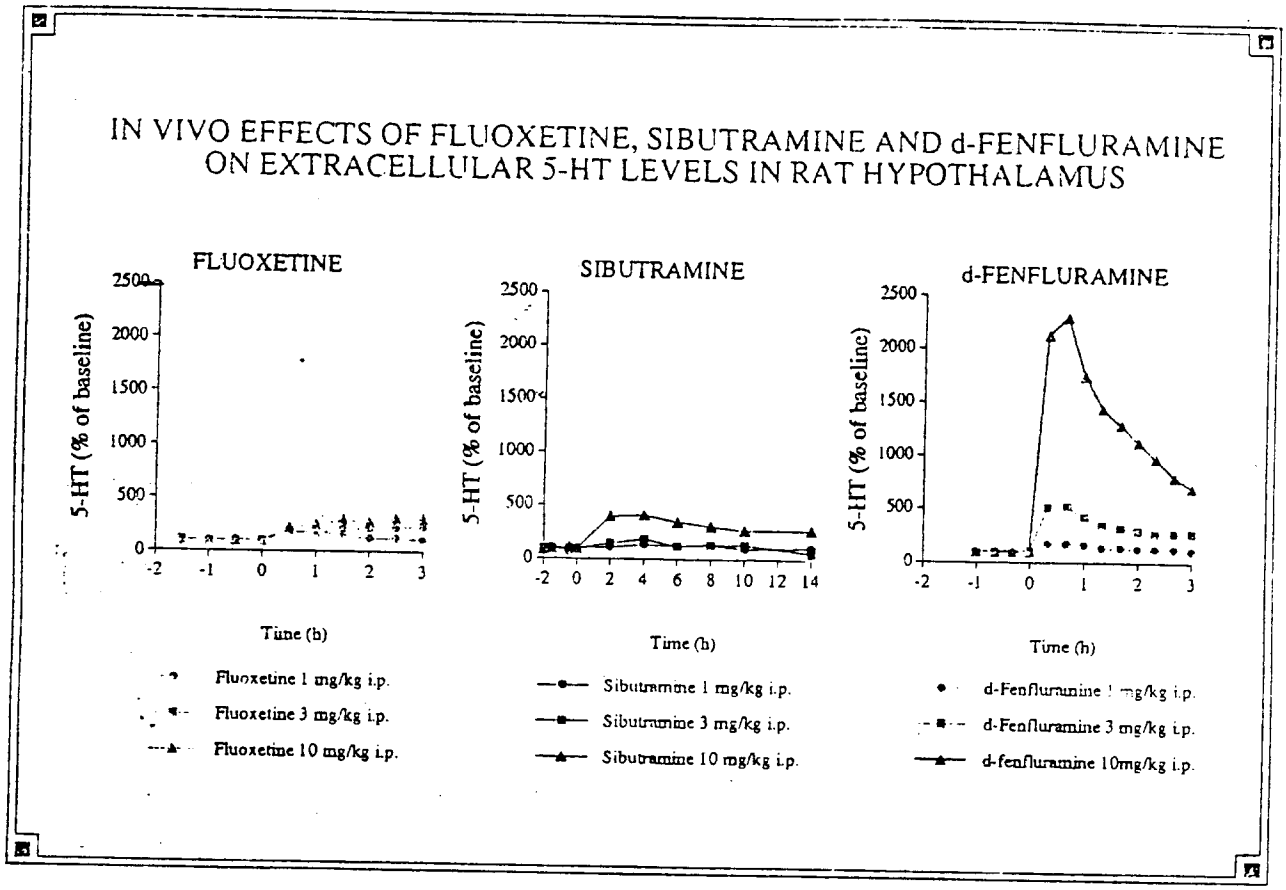
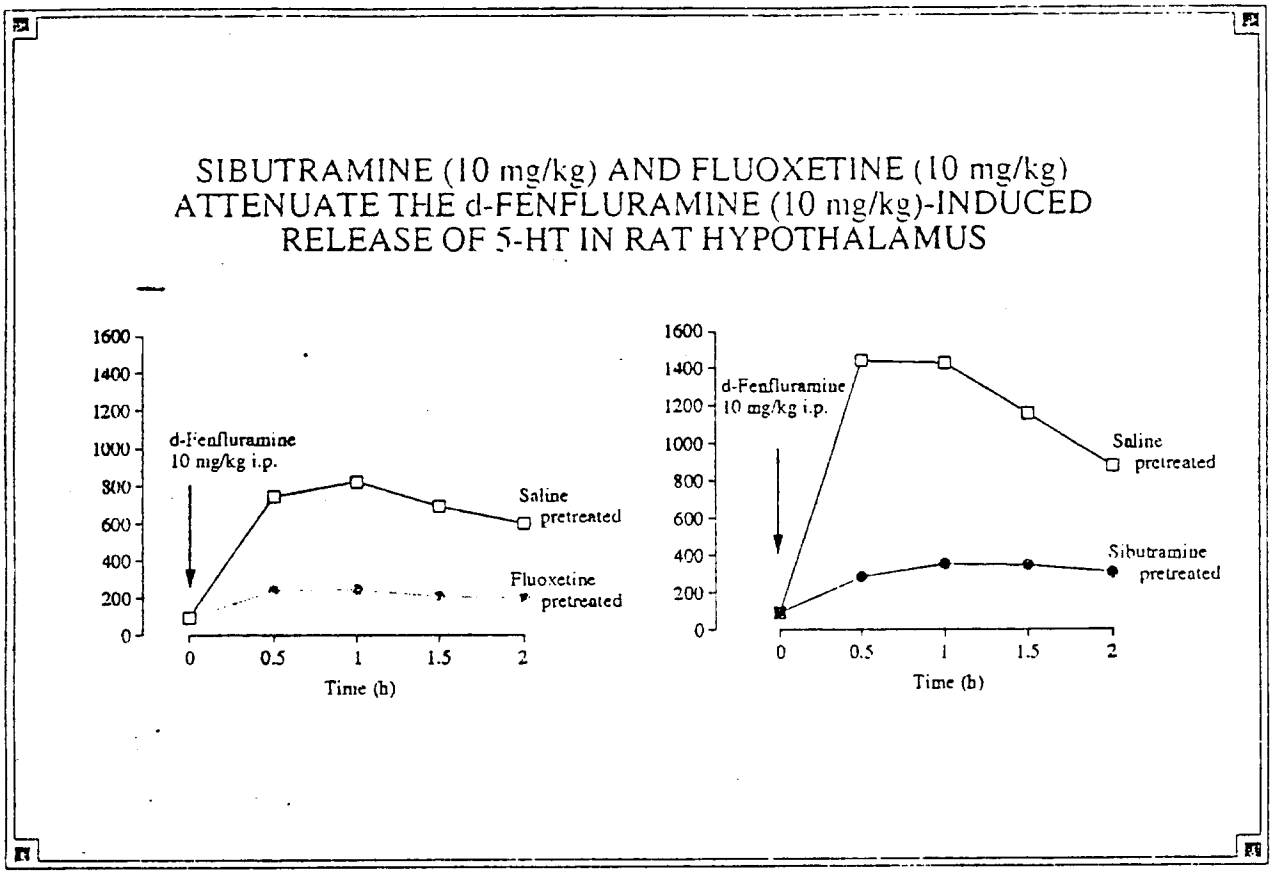
FIGURE 1.



Acute administration of pharmacologically effective doses (1-10 mg/kg) of the monoamine reuptake inhibitors, fluoxetine (5-HT-selective) and sibutramine (norepinephrine + 5-HT), produces small dose-dependent increases in hypothalamic extracellular concentrations of 5-HT, which are slow in onset.

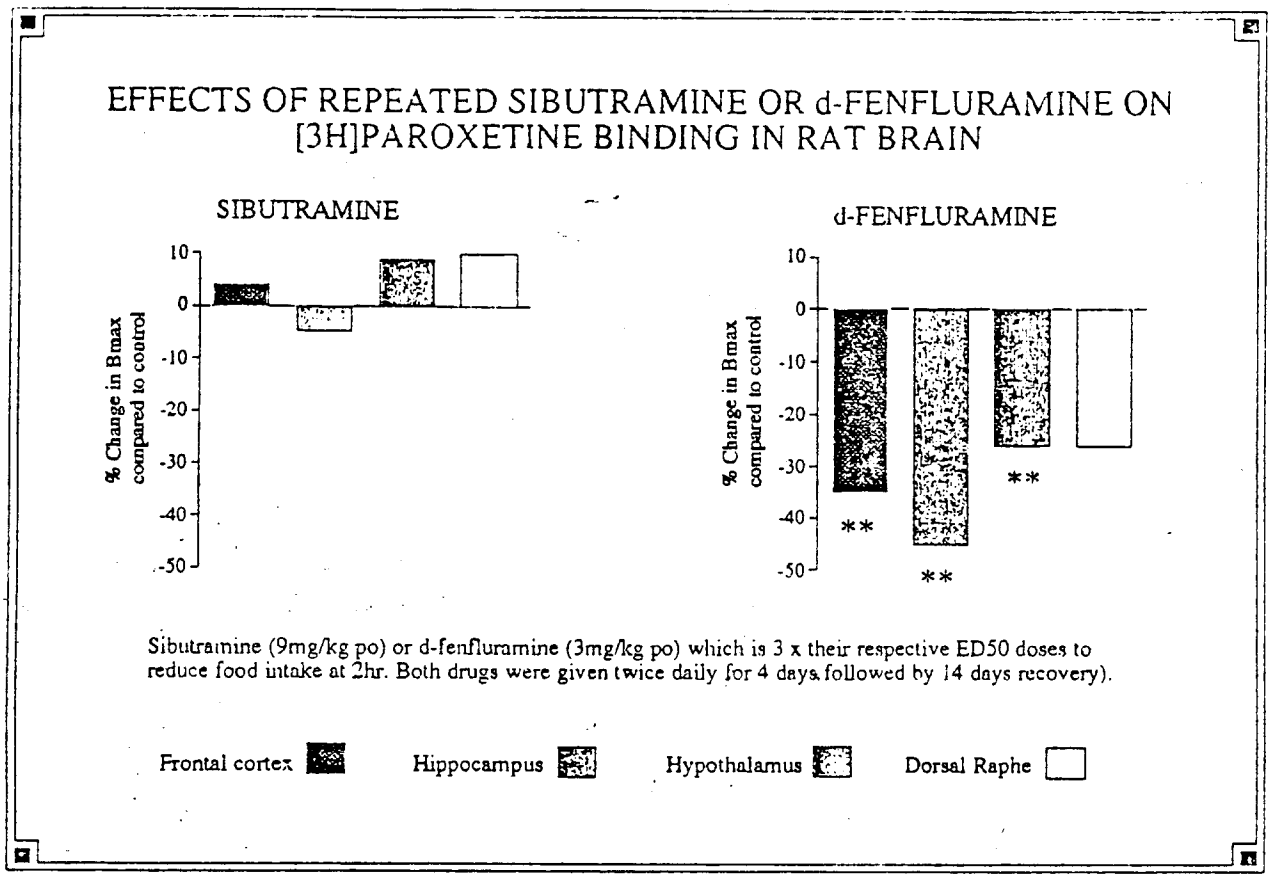
Despite sibutramine and d-fenfluramine being approximately equipotent as inhibitors of food intake, d-fenfluramine's effects on extracellular 5-HT concentrations are very different from those of sibutramine or fluoxetine. d-Fenfluramine which is predominantly a 5-HT-releasing agent, produces profound, dose-dependent increases in extracellular 5-HT concentrations that are rapid in onset and of relatively short duration.

FIGURE 2.



5-HT-releasing agents are taken into the nerve terminals by high affinity reuptake, where they displace 5-HT from storage pools. Pretreating rats with the monoamine reuptake inhibitors, fluoxetine (5-HT-selective) or sibutramine (norepinephrine + 5-HT), blocks these 5-HT reuptake sites and prevents the releasing-agent, d-fenfluramine, from entering the nerve terminal, thereby blocking its effects on extracellular 5-HT concentrations.

FIGURE 3.



The high affinity 5-HT reuptake site is an important mechanism for maintaining neuronal stores of 5-HT and it is an index of presynaptic 5-hydroxytryptaminergic neuronal viability.

Repeated administration of sibutramine at 3x its ED_{50} to inhibit food intake has no effect on the number of 5-HT reuptake sites labelled with [^{3}H]paroxetine. This lack of neurotoxicity is consistent with sibutramine's mechanism of action being 5-HT reuptake inhibition.

In contrast, d-fenfluramine when given repeatedly at the equivalent dose profoundly decreases the number of 5-HT reuptake sites in frontal cortex, hippocampus and hypothalamus; all brain regions innervated by 5-hydroxytryptaminergic neurones from the dorsal raphe. The demonstration of neurotoxicity with d-fenfluramine is consistent with its pharmacological action being predominantly 5-HT release.

The concentration of test compounds inhibiting 50% n
5-HT and dopamine uptake in vitro

Treatment	IC50 value (μM)		
	noradrenaline	5-hydroxytryptamine	do
BIS 54 524	2.2 ± 0.38	477 ± 111	11
amitriptyline	0.9	2.1	
clomipramine	1.6	0.6	
dothiepin	1.5	4.6	
imipramine	0.7	2.1	18
nomifensine	0.18	>10	0

The effect of standard compounds and BIS 54 524
using depletion of noradrenaline and dopamine b
by p-chloroamphetamine

Drug	ED50 $mg.kg^{-1}$ p.o		
	Noradrenaline	Dopam...	5-HT
Cianopramine	43	56	2.5
Citalopram	>100	>100	5.0
Fluoxetine	>100	>100	1.5
Panuramine	>100	>100	2.4
Zimeldine	>100	>100	0.6
Desipramine	20	> 30	>100
Imipramine	20	>100	>100
Nomifensin	2	> 30	>100
BIS 54 524 (earlier experiment)	15	33	13
BIS 54 524 (later experiment)	15	42	2

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